TOWARD A SUPERIOR FUTURE:
Advancing Science for a Sustainable Environment

Society of Environmental Toxicology and Chemistry
North America 38th Annual Meeting

12–16 November 2017 | Minneapolis, Minnesota

ABSTRACT BOOK

SETAC North America 38th Annual Meeting

TABLE OF CONTENTS

Platform Abstracts

Poster Abstracts

Indices

This book comprises the abstracts of the presentations for the platform and poster sessions of the Society of Environmental Toxicology and Chemistry (SETAC) North America 38th Annual Meeting, conducted at the Minneapolis Convention Center from 12–16 November 2017 in Minneapolis, Minnesota. The abstracts are reproduced as accepted by the Scientific Program Committee and appear in numerical order.

In each abstract, the presenting author's name is underlined. The author index cross-references the corresponding abstract numbers. Affiliation, session and keyword indices are also included.

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ABOUT SETAC

The Society of Environmental Toxicology and Chemistry (SETAC), with offices in North America and Europe, is a non-profit, professional society established to provide a forum for individuals and institutions engaged in the study, analysis and solution of environmental problems, the management and regulation of natural resources, environmental education, and research and development.

Specific goals of the society are:

- Promote research, education and training in the environmental sciences
- Promote the systematic application of all relevant scientific disciplines to the evaluation of chemical hazards
- Participate in the scientific interpretation of issues concerned with hazard assessment and risk analysis
- Support the development of ecologically acceptable practices and principles
- Provide a forum (meetings and publications) for communication among professionals in government, business, academia and other segments of society involved in the use, protection and management of our environment

These goals are pursued through the conduct of numerous activities, which include:

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- Publish scientific journals, a newsletter and special technical publications
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Neonicotinoid Insecticides – Potential Impacts on Non-target Organisms and Ecosystems

1 Assessing the distribution of neonicotinoids across Minnesota's Prairie Pothole Region and their potential effects to aquatic invertebrates

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The use of neonicotinoid pesticides is widespread throughout agricultural regions, including the Prairie Pothole Region of North America. In recent years, there have been growing concerns regarding the use of these pesticides and their impacts to non-target organisms, particularly bees and other pollinators. Neonicotinoids, being highly water soluble, have been found to occur widely in wetlands within the Prairie Pothole Region, and may also have critical impacts on these ecosystems, particularly through their potential impacts on aquatic insects. Prairie pothole wetlands are important ecological resources, and are best known for producing over half of North America's duck populations. Understanding the impact and fate of neonicotinoids will be important for effective management. To investigate the potential impact of neonicotinoids to prairie wetlands, we conducted a survey to assess the distribution and concentrations of neonicotinoids in a set of wetlands in western Minnesota. We coupled these surveys with an outdoor mesocosm experiment to investigate the effects of multiple pulses of a neonicotinoid on the abundance and diversity of emerging Chironomidae species. We sampled a set of 40 wetlands located within Waterfowl Production Areas (WPAs) on the US Fish and Wildlife Service's Morris Wetland Management District in Minnesota. Wetlands were selected to span a gradient of intensity of agricultural-use within their catchments and were sampled three separate occasions throughout the early part of the growing season. Despite previous reports of widespread detections of neonicotinoids in prairie wetlands, our initial water samples from most WPAs were below detection for all neonicotinoids, suggesting that non-agricultural buffer regions surrounding wetlands on WPAs may provide critical protection. To assess what the potential impacts of neonicotinoids could be, we conducted a mesocosm experiment using twenty 1200 L tanks, separated into four different treatment types to simulate pulsed additions of the pesticide via a rainfall event. Imidacloprid was added at .2, 2 and 20 µg/L to each of five replicate tanks, leaving five tanks to serve as control tanks. Preliminary analysis indicates that total chironomid emergence was significantly reduced in both the 2 and 20 µg/L treatments compared to control tanks, but no significant difference was observed between the lowest level treatment (0.2 µg/L) and controls.

2 Neonicotinoids in tree foliage: A risk for aquatic organisms?

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Neonicotinoids represent one of the most widely used insecticide classes worldwide. Their tremendous success is, amongst others, attributed to their systemic properties facilitating their rapid uptake and distribution within treated plants. Due to their high water solubility and high environmental persistence (in soil and plants), neonicotinoids may enter streams not only via surface runoff and spray drift but also within neonicotinoid-contaminated plant parts (e.g., leaves). There, particularly leaf-feeding detritivorous invertebrates may be affected through waterborne exposure and the consumption of contaminated leaves. In this context we performed a series of experiments, targeting in a first step the dose-dependent uptake of neonicotinoids into leaf material of black alder trees. These leaves were offered as food to shredders and allowed for the establishment of a dose-effect-relationships between neonictotinoids leaching form the leaf material into aquatic systems and the feeding activity of the organisms. By comparing this scenario with an ordinary waterborne exposure design (i.e. spiking of the water phase with the test item), a first indication for the relevance of the different exposure pathways was developed. In a follow-up experiment, the key leaf-shredding invertebrate *Gammarus fossarum* (Amphipoda) was subjected to these paths employing a two-factorial design for 21 days. Black alder leaves containing environmentally relevant amounts of the neonicotinoid thiacloprid (THI; ~250 µg/g) were used to expose *Gammarus* either a) towards THI leaching from contaminated leaves into the water (= waterborne path), b) via the consumption of THI-contaminated leaves (= dietary path) or c) a combination of both (= combined path). Both the waterborne and diet-related exposure pathways reduced gammarids' leaf consumption and lipid content when considered individually compared to a neonicotinoid-free control. The effects were more pronounced when subjected to a combination of both exposure pathways. Based on these results, adverse implications on the gammarids' physiological fitness and their role in the leaf decomposition process might be expected through the consumption of neonicotinoid-contaminated leaves even if concentrations in the surrounding medium are negligible. Consequently, neglecting dietary exposure might underestimate the environmental risk systemic insecticides pose for ecosystem integrity.

3 Impacts of neonicotinoid seed-treatments on aquatic invertebrates in Missouri wetland ecosystems

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Neonicotinoid insecticides are commonly used as seed-treatments on major agricultural row crops (e.g., corn). However, neonicotinoids are also highly persistent with long half-lives in soils (e.g., clothianidin: 1386 d). Due to their high solubility, neonicotinoids are readily transported in surface runoff into freshwater ecosystems such as rivers and wetlands. Previous field studies have documented neonicotinoid persistence in global surface waters as well as lethal and sub-lethal responses by aquatic invertebrates in laboratory settings; however, less is known about neonicotinoid occurrence in managed public wetlands of Missouri. We investigated the response of invertebrate communities to direct application of neonicotinoids in managed wetland ecosystems. In 2016, 22 wetlands from nine conservation areas were sampled for water, sediment, and aquatic invertebrates during spring (pre-wetland drawdown) and fall (post-wetland flood-up). During the summer, portions of wetland areas were planted with either untreated corn (control) or corn treated with a neonicotinoid (i.e., thiamethoxam). Using a series of linear mixed effects models, we evaluate water quality parameters and pesticide concentrations on aquatic macroinvertebrate metrics such as taxa diversity, morphology (e.g., size of individual), and production (e.g., abundance)). Water and sediment samples were analyzed for the six most common neonicotinoids (and fungicides) with 68% of wetland water in spring having low, but detectable clothianidin residues (mean: 0.006 µg/L; max: 0.012), whereas 10% of wetlands contained imidacloprid (max: 0.08 µg/L). In addition, 30% of wetland sediment samples contained a neonicotinoid (mean: 0.22 µg/kg; max: 2.5) with 65% of sediment containing detectable fungicide residues (mean: 0.15 µg/kg; max: 0.794). With sediment concentrations in spring being orders of magnitude higher than water, these pesticides may be persisting through the growing season and resolubilized during fall flood-up. Preliminary results indicate an overall decrease in diversity as well as decreased abundance and size of benthic organisms with increasing concentrations of neonicotinoids in the water and sediment. This has important implications for aquatic invertebrates as well as wetland-dependant species (e.g., waterfowl) as some concentrations, although below regulatory limits, may be impacting these wetland ecosystems.

4 Investigating the Cumulative Toxicity of Imidacloprid, Clothianidin, and Thiamethoxam to Aquatic Insects under Laboratory and Field Conditions

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Due to their widespread presence in global surface waters and their potential to cause adverse impacts on non-target invertebrate species, neonicotinoid insecticides have recently come under international scrutiny. Current research and regulation has primarily focussed on the toxicological effects of individual compounds. However, neonicotinoids are frequently detected in aqueous systems as binary and ternary mixtures. Therefore, this research aimed to characterize the cumulative toxicity of binary and ternary mixtures of select neonicotinoids (imidacloprid, clothianidin, thiamethoxam) to determine if single-compound chronic toxicity can be used to predict mixture effects in sensitive aquatic insects. Cumulative toxicity was first characterized in chronic (28-d) laboratory studies, using *Chironomus dilutus* as a test species and cessation of successful emergence as an endpoint. Preliminary assessments of single compound toxicity (EC_{50}) were used to develop parametric models, which were statistically compared to the toxicity of binary and ternary mixtures from similar laboratory studies, using a regression-based approach (MIXTOX model). Results indicated that under chronic exposure scenarios, the toxicity of neonicotinoid mixtures cannot be predicted using the common assumption of additive joint activity, as most mixtures display statistically significantly deviations from concentration-additive toxicity. Cumulative toxicity was further evaluated under semi-controlled field conditions, using limnocorrals (*in-situ* enclosures) installed in an experimental pond (St. Denis, Saskatchewan). Indigenous aquatic insect communities were exposed to either single compounds or binary neonicotinoid mixtures at equivalent dose-levels (28 d ΣEC_{50} for *C. dilutus* emergence) for 56 days. Insect emergence was monitored over the course of the exposure period, and compared between single compound and mixture treatments. Results obtained will allow for an assessment of whether neonicotinoid mixtures display similar deviations from concentration addition under more environmentally realistic exposure paradigms, whether neonicotinoid mixture toxicity can be effectively predicted from laboratory studies, and what risk these mixtures may pose to natural aquatic insect communities.

5 Analysis and Conclusions from USEPA's Neonicotinoid Preliminary Bee Risk Assessments

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In 2016-2017, the United States Environmental Protection Agency (USEPA) issued Preliminary Bee Risk Assessments for the neonicotinoid insecticides, imidacloprid, clothianidin, thiamethoxam and dinotefuran. The conclusions from these four preliminary assessments are summarized and compared with respect to direct contact and dietary-based risks at the individual and colony levels from agricultural uses of these chemicals. Although the focus in these documents is for honey bees, consideration of potential risk to non-A*pis* bees is also evaluated. Default exposures were based on the BeeREX model for all uses, and refined dietary exposures were based on empirical pollen and nectar residue concentrations from studies in which a single neonicotinoid was applied to a crop. Exposures of thiamethoxam and imidacloprid accounted for their relatively toxic metabolites, while clothianidin and dinotefuran were considered individually. For dietary risks to individual bees, most use patterns posed potential on-field risk for one or more honey bee castes, except for some seed treatments (e.g., oilseed crop group). On-field risk was assumed to be low for crops harvested prior to bloom or known to be unattractive to bees. Regarding off-field risks, foliar applications for all uses resulted in risks to individual bees at distances >1000 feet from the edge of the field. At the colony level, the risk assessment utilized semi-field Colony Feeding Studies (CFS) to establish endpoints based on consumption of treated sucrose solutions over an extended period of time. The thiamethoxam and clothianidin risk assessments developed a separate method to integrate both pollen and nectar exposures for evaluating colony-level effects based on measurements of residues in bee bread from the CFS. Exposures following foliar applications were generally more likely to result in colony-level effects than exposures made following soil applications. In general, colony-level dietary-based effects from seed treatments are not anticipated for A*pis* bees (e.g., honey bees).

6 Impacts of neonicotinoid seed-treatment use on non-target native pollinator abundance and diversity in Missouri agroecosystems

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Pervasively used as seed-treatments, neonicotinoid insecticides are widely applied across North American agroecosystems. Due to their high water solubility, neonicotinoids may be rapidly transported to adjacent field margins during precipitation events. Additionally, previous research demonstrates the potential accumulation of residues by non-target plant species. Unlike honeybees, numerous wild bee populations nest in the ground in close proximity to cultivated fields and flower foraging areas. To that end, it is unknown if native bee species are equally exposed to neonicotinoids through soil and non-target plants surrounding cropped fields (i.e., field margins). Few studies have evaluated neonicotinoid impacts on wild pollinator populations, including solitary and eusocial bee species (e.g., bumblebees). To evaluate the effects of neonicotinoid exposure on native pollinator abundance and diversity, we sampled 24 agricultural fields (treated and untreated) on four conservation areas in central and northern Missouri from pre-seeding to harvest in year 2016. At each field, we collected field and field-margin soils, sampled herbaceous and woody flowering species in field margins, and surveyed and collected a wide variety of native pollinators over time. Neonicotinoid residues were detected in field and field-margin soils during all sampling periods (frequency: pre-seeding, 58%; post-seeding, 67%; mid-growing, 69%; and, harvest, 58%). Clothianidin was the most-frequently detected active ingredient in field and margin soils with concentrations ranging from 0.16 to 55.7 μg/kg. Compared to untreated reference fields, native bee abundance was significantly less in both treated corn ($\beta = -0.72 \pm 0.20$, $P = 0.002$) and treated soybean fields ($\beta = -0.95 \pm 0.28$, $P = 0.005$). Here, we present our preliminary findings and discuss how this research improves our understanding of the potential impacts of neonicotinoid seed-treatment use on non-target native pollinator communities in agroecosystems.

7 Field Studies of Risk of Imidacloprid to Birds in North America

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Imidacloprid, the first neonicotinoid commercially developed, was recognized as significantly less toxic to birds and applied at significantly lower rates than most alternative insecticides in widespread use at the time it was developed; however, there was a question about possible risk to birds. Seed treatment and broadcast applications to turf grass were identified as the worst-case exposure scenarios based on expected concentrations in or on avian food items. Risk to birds from application to seeds of broad acre crops was evaluated in a series of cage and llight pen experiments conducted by the USDA National Wildlife Research Center in Gainesville, FL. These studies found that because imidacloprid was higly repellent, risk to birds is low. Even when given *ad libitim* access to a favorite food (rice seeds) treated with imidacloprid, red-winged blackbirds stopped eating before ingesting a debilitating dose. In the more than 20 years of field use in North America, there are no documented bird kill incidents from use of imidacloprid as a seed treatment. To evaluate the risk to birds from broadcast applications to turf grass, a large-scale field study was conducted at 8 golf course sites in which survival of ground-feeding birds was monitored using radio-telemetry, color bands, and intensive carcass searching. Survival of marked birds, including 500 radio-tagged American robins, brown thrashers and blue jays, was not significantly different between imidacloprid treatment and untreated control sites. Remains of many dead birds and other terrestrial vertebrates were detected during carcass searches, many being nestling/fledgling birds, but there was no significant difference in their number between treatment and control sites, and no indication from residue analysis that any of these mortalities was attributable to imidacloprid poisoning. After more than 20 years of commercial use, there are no documented bird mortality incidents from imidacloprid use on turf grass. Finally, bird population trends, as revealed by the North American Breeding Bird Survey, have generally increased since imidacloprid was introduced to the market in the mid 1990's. In particular, insectivorous birds which should be most vulnerable to indirect effects of insecticide use have increased in the corn belt and prairie pothole regions where neonicotinoid use is greatest.

8 Neonicotinoids on the landscape: Evaluating avian exposure to treated seeds in an agricultural region

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Neonicotinoid pesticides (e.g., imidacloprid, thiamethoxam, clothianidin) are commonly applied to agricultural seeds such as corn, soybean, and wheat. Neonicotinoid-treated seeds could be available to wildlife through spillage during planting or through exposed seeds near or at the soil surface after planting. We are examining exposure of wild birds to these pesticides in agricultural landscapes of Minnesota. We are quantifying seed availability at the soil surface in recently planted fields and the rate of seed spills during planting, as well as documenting wildlife eating treated seeds with trail cameras. In the images and field observations to date, we have documented numerous gamebirds and non-game birds, as well as mammals such as white-tailed deer (*Odocoileus virginianus*), rodents, lagomorphs, and raccoons (*Procyon lotor*), consuming treated seeds. We are also conducting laboratory experiments on domestic chickens (*Gallus gallus domesticus*), which are taxonomically similar to grouse and pheasants, to identify sampling methods to assess exposure in field settings. Early results suggest that residues after dosing are highest in the brain, followed by liver, spleen, muscle, blood, kidney, and then feces in birds. Residues were consistently detected in fecal samples for several weeks after exposure and thus fresh feces provide a reliable, non-invasive indicator of recent exposure. Twenty-two of 34 (65%) greater prairie-chicken (*Tympanuchus cupido*) fecal pellets and 47 of 56 (84%) sharp-tailed grouse (*Tympanuchus phasianellus*) pellets collected from leks had detectable concentrations of at least one neonicotinoid, with imidacloprid being most commonly detected. Hunter-harvested samples offered another alternative to examine recent exposure in wild populations; 18 of 27 (67%) greater prairie-chicken livers and 41 of 46 (89%) sharp-tailed grouse livers contained detectable concentrations of at least one neonicotinoid. Our study is ongoing, but we encourage other researchers to initiate studies to determine exposure in wild populations.

Advances in Environmental Fate and Exposure Modeling

9 Advances on reactive transport modeling: Modeling adsorption of heavy metals on iron oxides using an innovative surface complexation model

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Predicting the fate and transport of contaminants in the subsurface is of utmost importance in terms of risk assessment. Reactive transport models account for the physical and geochemical processes that control the movement of inorganic contaminants in the environment, including adsorption which is one of the most significant processes, especially for the transport of heavy metals. Iron oxides are highly reactive surfaces and various models have been developed to describe the reactivity of various phases, such as goethite and ferrihydrite. Adsorption has been traditionally described using empirical relationships, such as distribution factors (Kd) and Frendlich isotherms, that, however, do not account for the impact of variable chemical conditions on adsorption. A more robust description of adsorption is provided by surface complexation models (SCMs) that simulate adsorption mechanistically under various conditions. SCMs have been proven superior tools to estimate the partitioning of ions in the solid – solution interface. However, there are still two main limiting factors associated with the application of SCMs: a) a significant number of parameters is required, and thus their application is limited to pure mineral studies, and b) the parameters are interdependent with each other and cannot easily estimated experimentally, and therefore are usually fitted or adjusted to describe adsorption data, exhibiting a great variability among different studies. In this study, we propose a SCM tied with a multi - start global optimization algorithm to estimate the fitted parameters and predict iron oxides reactivity. This tool enables the simultaneous optimization of different parameters revealing unified parameters that were previously scattered by the interference of other variable parameters. Chromate was used as a model contaminant in this study, however, this approach can be easily extended to any inorganic compound. Specifically, a 3–site model was used to describe surface protonation using site densities derived from the structure and morphology, and protonation constants derived from the literature or fitted to mineral-specific charging curves. For chromate adsorption, insights from spectroscopy and batch adsorption experiments are incorporated to build the model. Spectroscopy profiles for chromate surface complexes were used to extract the inner – sphere equilibrium constants while a sensitivity analysis was performed for the batch adsorption data.

10 Predicting Non-linear Adsorption of Ionogenic Species to Organic Carbon Using Quantum-chemically Estimated Abraham pp-LFER Solute Parameters

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Procedures for accurately predicting linear partition coefficients for neutral chemicals onto various sorbents (e.g., black carbon, natural organic matter, clay) are reliable and well-established. While these methods have been extended to include charged species with some success, non-linear partitioning has largely been ignored. Previously, an accurate method for modeling non-linear adsorption of neutral species onto various black carbons with a single, sorbate-specific, binding parameter – the lognormal Langmuir (LNL) isotherm – was developed. The purpose of this paper is to present a procedure for predicting non-linear isotherm parameters of charged species, specifically the median Langmuir binding constants, K_L, from molecular structure only. An Abraham poly-parameter linear free energy relationship (pp-LFER) is able to predict median Langmuir binding constants for a set of primary, secondary, tertiary, and quaternary amines sorbed onto soil organic carbon using quantum-chemically estimated Abraham (QCAPs) parameters for the charged species. Predictions made using the QCAP solute descriptors were then compared to predictions made using Absolv-estimated Abraham solute parameters (AAPs) for the corresponding neutral species. For a set of 76 amine cations, QCAP predictions of organic carbon – water partition coefficients performed similarly to those made with the neutral species AAP descriptors (RMS errors of 0.543 and 0.547, respectively). Finally, non-linear experimental isotherms were reproduced using QCAP descriptors with an RMS error (RMSE = 0.413) comparable to those obtained for linear partitioning of neutral organic species.

11 Co-deployment of silicone equilibrium passive samplers for multicompartment chemical transport modeling using silicone activity coefficients

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A wide range of equilibrium passive samplers have been developed and applied for the measurement of hydrophobic organic chemicals (HOCs) in different environmental compartments. Converting measured polymer concentrations to chemical activities or fugacities for the deduction of pollutant transport along chemical gradients remains however challenging. This is not least because accuracy of such measurements largely depends on (i) the degree of equilibrium attained between passive sampler and matrix, (ii) the methods used to assess the degree of equilibrium and (iii) the quality of polymer-water partition coefficients that are used to convert measured polymer concentrations to freely dissolved concentrations. We therefore followed a passive sampling strategy where one and the same polymer was equilibrated in different environmental compartments (air-water-sediment) with the goal to link measurements on the basis of polymer activity coefficients. We deployed ultrathin silicone fast equilibrating membranes in various thicknesses (1, 20 and 50 µm) and confirmed equilibrium through linear regression of analyte mass vs. polymer volume. In addition, we spiked the passive samplers with performance reference compounds (PRCs) as a secondary method to assess the degree of equilibrium. Silicone membranes were calibrated and applied for equilibrium sampling of a wide range of HOCs (i.e. polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), hexachlorocyclohexanes), in the air, water, sediment and deep sediment at an industrial site in Norway and Sweden. The choice of using the same type of polymer in all environmental matrices and limiting the protocol to a single analytical methodology showed to be very effective in reducing the uncertainty associated to the chemical analysis. Moreover, this strategy allowed estimating the chemical activity of HOCs across compartments from a single equation: a = γ_silicone * C_silicone, where a, γ_silicone and C_silicone is the chemical activity, the activity coefficient and the concentration in the silicone, respectively, circumventing the use of multiple and sometimes uncertain partition coefficients (polymer-water and polymer-air), and hence reducing uncertainty. This approach is thought to reduce the uncertainties associated with multicompartment chemical transfer modelling and allows for a thermodynamically based assessment of contaminant gradients in complex real-world polluted environments.

12 Impacts of ocean circulation on biologically relevant PCB residence times in marine environments

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Polychlorinated biphenyls (PCBs) bioaccumulate in food webs and have been associated with a wide range of health effects including cancer, neurotoxicity, and endocrine and thyroid disorders. Their ban from production in the U.S. in 1979 and inclusion in the Stockholm Convention in 2004 has greatly reduced primary emissions. However, PCBs are still detectable throughout marine environments, including polar regions due to their long-range transport. Here we present a new simulation for PCB cycling in the global oceans within a 3-D ocean general circulation model (MITgcm). The simulation is forced by atmospheric inputs from the GEOS-Chem global atmospheric model based on previously published historical emissions data. We use the model to investigate how ocean circulation, biological productivity and particle export fluxes affect residence times in biologically relevant regions. The model agrees well with available observations and shows seawater concentrations of PCB-28, PCB-101, PCB-153 and PCB-180 have declined by less than 60% in the Arctic Ocean compared to a mean decline of over 80% in the North Atlantic since 1980. This leads to sustained exposures of marine organisms and individuals in northern regions. We find that currently more than 90% of PCBs can be found below 1000 m and ~40% of PCBs are located in ocean basins in the Southern hemisphere. Already, in two of the nine major ocean basins diffusive evasion from the ocean to the atmosphere exceeds wet and dry deposition from the atmosphere onto the ocean's surface. Globally, we estimate that the oceans emit 40 t of PCB-28, PCB-101, PCB-153 and PCB-180 every year, thereby significantly adding to PCBs from primary releases in remote locations. The importance of the deep ocean as a source of PCBs to biologically relevant ocean layers, as well as of the surface ocean as a source of PCBs to the atmosphere will likely increase in the future.

13 Probabilistic methods to address uncertainty in pesticide use at the watershed scale and impact on aquatic resources

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The use of probabilistic methods to address the uncertainty associated with the spatial and temporal distribution of pesticide applications was evaluated in the Zollner Creek watershed located in the Willamette River Basin in Oregon. The Zollner Creek watershed contains designated critical habitat for two ESA listed Pacific salmonid species and has been intensively monitored since the early 1990s. The Soil and Water Assessment Tool (SWAT), a watershed scale ecohydrologic model, was parameterized to simulate pesticide transport in the Zollner Creek watershed. Probabilistic methods were developed to randomize pesticide application patterns at the field scale based on local knowledge of pesticide use practices. Coupling the probabilistic approach to randomize pesticide application patterns with the SWAT model, a range of aqueous pesticide concentrations can be estimated based on realistic pesticide application patterns on a daily time step. The SWAT estimated range of pesticide concentrations were compared with monitoring data for frequently detected herbicides and insecticides. Pesticide concentration ranges and patterns of exposure were used to evaluate both acute and chronic impacts on salmonids and their food web.

14 Conditional Simulation as a Tool for Incorporating Uncertainty into Exposure Concentration Predictions at Contaminated Sediment Sites

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Contaminant fate and transport (CFT) models are commonly applied at contaminated sediment sites to predict exposure concentrations under various remedial strategies. Although these models have become more sophisticated in process representation and spatial resolution, the initial distribution of contaminant concentrations in surface sediments remains a key source of input uncertainty. Model initial conditions are typically based on an interpolated map of contaminant concentrations developed from data that are sparse relative to the resolution of the numerical grid, and predictions of exposure concentrations may be sensitive to the mapping technique employed. This is particularly true in the evaluation of remedial alternatives in which target areas are based on a "Remedial

Action Level" (RAL; a threshold concentration). Accurate assessment of mean concentration reductions due to targeting distinct areas requires realistically representing both the distribution of concentrations and their spatial structure. This work uses the geostatistical technique of conditional simulation (CS) to account for uncertainty by providing multiple equally plausible concentration maps that reproduce the distribution and structure of the underlying data. The resulting maps are needed to initialize CFT model calibration and remedial alternative projection simulations for the Lower Passaic River, a portion of the Diamond Alkali Superfund site presently undergoing a Remedial Investigation and Feasibility Study. Three strategies were investigated for establishing a tractable and consistent basis for calibration and projection: 1) simulating all of the maps; 2) generating an expectation across all maps; and 3) applying selection criteria to choose a single map. Model run-times prohibit running all of the CS realizations through calibration and projection, and an expectation approach proved unwieldy for realistically representing a remedial design. Instead, criteria were developed to select a single suitable concentration map that provides consistency across calibration and remedial alternative simulation and incorporates uncertainty in pre- and post-remedy exposure concentrations.

15 Oil Spill Modeling for a Comparative Risk Assessment (CRA) of Response Options for a Deepwater Oil Release

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Subsea dispersant injection (SSDI) was used during the Deepwater Horizon spill, and is being considered to increase effectiveness of dispersant treatment over that achievable at the water surface; reduce human and wildlife exposure to volatile organic compounds (VOCs); disperse the oil into a large water volume at depth; enhance biodegradation; and reduce surface water, nearshore and shoreline exposure to floating oil. Potential tradeoffs include increased exposures to dispersed oil and dissolved components at depth. Blowout and oil spill model simulations of non-treated versus SSDI-treated deep water oil discharges in De Soto Canyon of the Gulf of Mexico were compared with respect to oil fate, amount and area of surfaced oil, and exposure concentrations above thresholds for potential effects. Simulations assuming no response and various response alternatives (i.e., SSDI in addition to mechanical recovery, in-situ burning, and surface dispersant application) were evaluated in a Comparative Risk Assessment designed to identify response strategies that minimize long-term impacts. The results showed that even with a substantial capacity of equipment applied, mechanical and ISB removed only a small fraction of the oil from this offshore release that would otherwise be floating or evaporate. Compared to cases without use of dispersant, SSDI: reduced the size of oil droplets by an order of magnitude, substantially decreased the amount of oil on the water surface and on the shoreline, increased dissolution and degradation rates of hydrocarbons at depth, increased weathering rate of rising oil such that floating oil contained much lower content of soluble hydrocarbons, decreased surface water concentrations of dissolved hydrocarbons, and decreased VOC emissions to the atmosphere and, therefore, reduced human and wildlife exposures to VOCs. The tradeoff was that with SSDI there was greater exposure to hydrocarbons in deep water. However, densities of biota are much lower in deep water than near the water surface, where sensitive early life history stages of fish and invertebrates are most abundant. This approach provides decision makers with quantitative environmental exposures with which they may evaluate risk tradeoffs regarding appropriate response strategies for mitigating impacts from oil and gas released during a deep water blowout.

16 Quantitative Assessment of the Transport of PCBs Emitted from Chicago's PCB Inventory to Lake Michigan

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We hypothesized that Chicago remains an ongoing source of airborne PCBs to Lake Michigan due to emissions of PCBs from both legacy and modern sources. To address this, the overall strategy employed by this study was to simultaneously sample air and water to measure PCB concentrations and determine the fate of PCBs in southern Lake Michigan. We successfully completed a detailed analysis of the quantity and transport of southern Lake Michigan's PCB content; developing a detailed mathematical air-water exchange model and coupling that with emissions and air dispersion modeling (AERMOD) to estimate the magnitude of the effect of Chicago's PCB inventory on Lake Michigan. Samples were collected (n=20) aboard the USEPA's R/V *Lake Guardian*, in September of 2010; 5 km off the coast of Chicago. Gas-phase ΣPCB concentrations ranged from 0.19 to 1.1 ng m^{-3} with an average of 0.75 ± 0.3 ng m^{-3}. Dissolved-phase ΣPCB concentrations in water ranged from 150 to 170 pg L^{-1} with an average of 160 ± 13 pg L^{-1}. As expected for the Aroclor history of the area, the distribution of congeners in air and water resembled a mixture of Aroclors 1242, 1248, and 1254. Air-water exchange was predicted though application of congener-specific physical-chemical properties, local meteorological data, and the congener-specific air and water concentrations. For the entire period of study, net ΣPCB fluxes were determined to range from -184 to 54.9 ng m^{-2} day^{-1} with an average of -58.3 ± 79.3 ng m^{-2} day^{-1}; congener specific net flux ranged from -14.3 to 5.56 ng m^{-2} day^{-1} for PCB3 and PCB11, respectively, where positive and negative fluxes indicate volatilization and absorption, respectively. A strong correlation between concentration and wind direction was found for ΣPCB, as well for 67 individual congeners ($R^2 = 0.69$-0.95, $p < 0.05$). Interestingly, all these individual congeners are present in Aroclor mixtures. These results suggest that Chicago remains a source of PCBs, primarily medium to higher chlorinated congeners (tetra-, penta- and so on), to Lake Michigan. Additionally, utilizing AERMOD with predicted emissions rates, we determined that approximately 20% of the gas-phase ΣPCB concentrations measured above Lake Michigan originated from Chicago.

Life Cycle Sustainability of Consumer Products

17 Concept to Practice: California Safer Consumer Product Alternative Analysis

X. Zhou, California Department of Toxic Substances Control

The California Department of Toxic Substances Controls released in June 2017 its Alternatives Analysis Guide (Version 1.0) as a resource reservoir of tools, methods, examples and a road map to help manufacturers conduct an Alternatives Analysis. The State of California's Safer Consumer Products (SCP) regulations require manufacturers of consumer products containing Chemical(s) of Concern to perform a comprehensive Alternatives Analysis to answer two fundamental questions systematically: 1) Is this chemical ingredient necessary? 2) Is there a safer alternative? The SCP regulations lay out the most systematic and expansive two-stage (screening and comprehensive) regulatory Alternatives Analysis framework meant to shift paradigm of consumer product design. The process involves a broad function-based cradle-to-grave approach and addresses trade-offs in and among technical performance, hazard, exposure, economic impacts, and life cycle impacts. Consideration of multimedia life cycle impacts in an Alternatives Analysis is an essential requirement looking beyond chemical-by-chemical substitution to avoid regrettable substitution and burden shifting. The presentation will explore the linkage between the recent developments in Life Cycle Assessment and regulatory Alternatives Analysis. This also includes an overview of pertinent Life Cycle Assessment concepts and steps applicable for performing Alternatives Analysis. Overall, Life Cycle Assessment promises a systematic way for developing and adopting appropriate models, tools, and strategies for Alternative Analysis to aid chemical policy decisions in the arena of reducing and eliminating hazardous ingredients in consumer products.

18 Rapid Assessment of Life Cycle Environmental and Human Health Impacts of Chemicals

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Chemicals in consumer products have been the foci of regulatory bodies recently, such as the reform of Toxic Substance Control Act at USEPA and the Safer Consumer Products Program in the State of California. Nevertheless, understanding the environmental and health impacts of chemicals remains to be a challenge. Everyday about 15,000 chemicals are added to the Chemical Abstract Service, while the speed at which our knowledge on their life cycle impacts grows is much slower. To address this challenge, we combine information technology, machine learning techniques with best available sciences in chemical release, fate and transport, exposure assessment, Quantitative Structure Activity Relationship (QSAR), and life cycle inventory analysis to develop a web-based platform – the Chemical Life Cycle Collaborative (CLiCC). On a screening level, CLiCC employs QSAR and Artificial Neural Network to provide hazard information and life cycle impact assessment results for chemicals, such as energy demand, greenhouse gas effects, ecotoxicity, human toxicity, etc. To improve on the existing life cycle inventory databases in terms of coverage and transparency, CLiCC uses a stoichiometric-based approach to construct cradle-to-gate life cycle inventory database for over 1,000 building block chemicals that can be further expanded upon. CLiCC assesses ecological and human health risks by interconnecting the use phase release, fate & transport, and exposure of chemicals. Use phase release considers the chemical's physical chemical properties, functional uses, product categories, and release factors in a systematic approach that requires little prior information on the chemical. The dynamic fate & transport model in CLiCC accommodates not only constant release of chemicals to the environmental but also seasonal releases or one-time accidental spills – scenarios where traditional models that rely on stead-state assumptions cannot be applied to. Combining this fate & transport model with comprehensive models for outdoor, indoor, and dermal exposures, both the long-term average level and exceedance over threshold levels of concern throughout years can be assessed. Uncertainty characterization using Monte-Carlo simulation in CLiCC provides better support for decision-making processes. CLiCC enables rapid, high-throughput screening of life cycle impacts for new chemicals and acts a platform for promoting life cycle thinking and practices?

19 A life cycle oriented method to assess toxicological potentials of product systems (ProScale)

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Increasingly, various stakeholders require information on human toxicity aspects of products beyond regulatory requirements for chemicals, e.g. in the framework of Life Cycle Assessment ("LCA"). The method developed (here referred to as "ProScale") aims to be a science-based, transparent, pragmatic and generally applicable methodology to compare hazard and exposure potential of products with a life cycle perspective. It is a development of initial ideas of an approach for merging applicable information from Risk Assessment ("RA") with LCA. The ProScale method has been developed in an industrial consortium with expertise both from the LCA and RA areas. Prerequisites for the method have been to: (i) assess the relevant direct exposure potential along the whole life cycle; (ii) use existing data, e.g. REACH based; (iii) allow comparison in relation to technical performance; and (iv) be relevant for business-to-business and business-to-customer communication. The ProScale method estimates a score for a specific instance of *exposure* for each substance for a given process and exposure route expressed in an Exposure Concentration Factor (ECF). The ECF is modelled based on the ECETOC Targeted Risk Assessment Tier 1 approach for both worker and consumer exposure. This is combined with its corresponding *hazard* for the substance considered expressed in a ProScale Hazard Factor (HF). The HF reflects the health hazard effect severity and potency based on Hazard statement Code(s) (H-phrases) and Occupational Exposure Limits (OEL). Then it relates this score to the amount of different substances in relation to the defined unit process at hand. Subsequently, the method accounts for the amount of each unit process necessary for the fulfilment of the functional unit as defined by the product system/flow chart. This is done for all included exposure instances, so that eventually a large number of ProScale scores for all included exposure instances are making up the ProScale toxicity impact potential for the overall product system of the studied product (PSP = ProScale of Product). A product can in this context also mean a service provided by the system. ProScale allows for comparisons based on the same function as for other LCA indicators and as part of product sustainability assessment. ProScale has been presented in SETAC Brussels (May 2017). Test cases realized in EU will be presented and the applicability in the US will be discussed.

20 High-throughput assessment of use-phase exposures to chemicals in building materials

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The use phase of products, generally ignored in traditional LCA, is essential to be considered when assessing the life cycle sustainability of consumer products, since there is growing evidence that chemical intakes during use phase could be as important as environmentally mediated exposures. The present study aims to quantify the use-phase exposures to chemicals in building materials in a high-throughput manner, which is expected to be a major emission source indoors. Based on the Pharos database of real building materials, 22 product categories (e.g. flooring, ceiling, insulation, etc.) and 180 chemicals were identified, giving 355 unique chemical-product combinations, for which chemical concentrations in products were obtained. To quantify chemical emissions from building materials, a parsimonious model which describes the diffusive emission of chemicals from materials and the loss by ventilation was used for VOCs, while a simplified model accounting for sorption on indoor surfaces was used for SVOCs. Use-phase exposures through inhalation, dermal contact, gaseous dermal uptake and dust ingestions were calculated using the Product intake fraction (PiF) metric - fraction of chemical in building materials that is taken in by humans during the entire use phase (15 yrs assumed). PiFs and intake doses were calculated for 1 adult and 1 child under 5 yrs old in a typical North American house. Results showed that inhalation is the dominant exposure pathway for VOCs in building materials, while dermal contact and dust ingestion are more important for SVOCs. The total PiF accounting for all exposure pathways ranges from 10^{-10} to 10^{-2} for SVOCs, and from 10^{-4} to 10^{-2} for VOCs. Children have higher dermal contact and dust ingestion PiFs than adults due to their higher dermal contact frequency and dust ingestion rates.

Among the various building materials, flooring products give highest total PiFs due to their higher potential for human contact and lower thickness. The intake doses, which combine PiFs and chemical concentrations in product, are higher for children than for adults, and the total intake dose for children ranges from 10^{-2} to 10^{6} µg/kg-d. This study demonstrates the method of performing high-throughput use-phase exposure assessment of chemicals in consumer products, and provides a database of use-phase exposure factors for building materials which can be further integrated into characterization factors and help improve LCA of consumer products.

21 Advancements in technologies often change previous norms for recycling strategies

W.L. Goodfellow, Exponent, Inc. / BioSciences Practice; Y. Masue-Slowey, Exponent, Inc.

Recent advancements in sorting and recycling technologies have allowed the effective recycling of products that were once thought as not viable candidates for recycling. These advancements now offer an opportunity to look at certain products in a different light, demonstrating that recycling strategies should be dynamic rather than static considerations. The consumer usage of expanded polystyrene (EPS) will be discussed in this paper as the case example to demonstrate the importance to routinely evaluating past strategies with technology advancements. EPS single-serve food containers have desirable properties that are highly valued in commercial and retail industries, especially for prepared meals. EPS protects food from moisture and has high thermal insulation capacity; therefore, EPS single-serve food containers are sanitary and help prevent food from spoilage. This case example will be compared against commonly utilized replacement products that have been used for single-serve food containers. EPS single-serve food containers are routinely recycled in many United States and Canadian communities as well as other countries; however, some communities have attempted strategies of product banning rather than environmentally effective recycling. Energy consumption is a critical measure of environmental impact and was used as one of the principal parameters to evaluate the environmental effectiveness of recycling EPS and most likely substitute products. Nearly three times as much energy is required to make a paper product as compared to EPS. Furthermore, less energy is consumed to recycle EPS products than to produce new EPS products from virgin material. Given consumers and retailers continued interest in the use of single-serve EPS products and the advancements of sorting and recycling technologies, recycling is preferable to not recycling, in terms of environmental impact.

22 Including Site-Specific Biodiversity Impacts in LCA using Big Data

T. Schultz, SCS Global Services / Corporate Sustainability Services

Many LCAs omit critical issues such as biodiversity and toxicity impacts. Finding suitable data to create site-specific assessments of these issues has been a constraint for many LCA practitioners. Today, there is now a wealth of reliable data publicly available to evaluate these impacts. Databases such as the US's Forest Inventory Analysis National Program and Canadian Forest Inventory provide site-specific information on carbon stock and vegetative composition of forests to evaluate biodiversity impacts related to wood and agricultural products. Additional databases can enable quantitative tracking of changes in ecosystem integrity around the world, helping identify individual species affected by different land use practices globally, as well as identify wood which is sourced from deforestation "hot spots." These "Big Data" sources enable LCAs to quantify biodiversity and toxicity impacts in a way that was not previously possible. In this session, Tobias Schultz will present some of the "Big Data" sources now publicly available, and one methodology that LCA practitioners can use to include biodiversity and toxicity impacts in their LCAs. Case studies presented will include: An LCA for Stella McCartney that evaluates the environmental performance of viscose fiber sources from several regions around the world, including an evaluation of ecosystem impacts using a mix of databases. This LCA, peer reviewed by an independent stakeholder panel, is the most comprehensive to date completed for viscose fiber. The yerba mate provider Guayakí used IPCC-published data on forest carbon stocks to estimate the amount of CO_2 stored in trees as a result of its funding restoration of thousands of acres of South American forests. The free, online life cycle assessment tool developed for the environmental non-profit Environmental Paper Network that enables companies, institutions, purchasers, and educators to measure and compare the sustainability of paper products. Attendees will leave the session having learned about these new data sources and tools, and how they can be used for LCAs applied at their own companies.

23 Nanoenabled textiles: A life cycle case study in environmental benefits and costs

A. Hicks, University of Wisconsin - Madison / Civil and Environmental Engineering

Nano-scale silver enabled textiles represent the next revolution in textile technology. The value of the market for nano-silver coatings and products is expected to grow from $290 million in 2011 to $1.2 billion worldwide by 2016. The major selling point of these products is that they will emit fewer odors than conventional products due to the antimicrobial properties of the silver, and thus, require less frequent laundering. Washing with respect to conventional textiles is the phase of the lifecycle with the greatest environmental impact. At the same time, the silver enabled products are more complex to manufacture, require additional raw materials that must be mined and refined, and release silver in various forms throughout the life cycle. The question then becomes whether the increased raw materials and manufacturing impacts will be greater than the potential savings through reduced laundering, and whether consumer use habits will change as function of adoption of these products. The results and implications of a cradle to grave midpoint lifecycle assessment (LCA) are presented, using three commercially available silver enabled textiles. This work utilizes both experimental and modeling techniques to draw conclusions as to the environmental impact of silver enabled textiles along with their antimicrobial benefits.

24 Quality Assurance in Life Cycle Studies through Independent Data Review and Responsible Database Management Practice Assessment

B.W. Vigon, Breveja Environmental Consulting LLC; G. Sonnemann, University of Bordeaux / The Life Cycle Group CyVi; A. Asselin, Consultant Life Cycle Thinking; D. Schrijvers, University of Bordeaux / ISM CyVi

The application of life cycle data, whether in formal life cycle assessments or in related life cycle-based approaches, is expanding globally. There is a critical need for ensuring the quality and completeness of information used in such studies as well as the responsible management of databases used to support these efforts, allowing data users to assess the fitness and suitability of information available. Under the auspices of the UNEP/SETAC Life Cycle Initiative, a multi-year effort to develop methods and tools for this type of QA effort has concluded. Several workshops and two rounds of implementaiton roadtesting have served to develop and refine criteria and rigorous procedures for conducting these evaluations. This presentation will provide results of those efforts as well as practical examples of how those results are being used by data providers, database managers, and end users. Ultimately, the goal is to deliver the results of the assessments through web-based portals and data network hubs being established to support the life cycle community. The real beneficiaries will be practitioners and decision-makers who will have greater understanding the quality of the informaiton used and enhanced credibity of life cycle approaches overall.

Food Web Models – Lowering Uncertainties in Predictions at Contaminated Sediment Sites

25 The use of passive sampling devices to help implement the new USEPA bioaccumulation model to protect human health

D. Shea, T. Johnson, North Carolina State University; S.L. Mort, North Carolina Department of Environmental Quality / Division of Waste Management; X. Xia, X. Kong, North Carolina State University

The USEPA recently updated ambient water quality criteria (WQC) for human health with a new method to predict the bioaccumulation of 94 chemicals in aquatic food chains. The method uses octanol-water partition coefficients (Kow) for each chemical along with a national database of measured bioaccumulation at different trophic levels to obtain a national food chain multiplier (FCM). This FCM is then combined with site-specific environmental parameters to predict bioaccumulation at different trophic levels of the food chain. Polychlorinated biphenyls (PCBs) are among the 94 chemicals included in the new EPA method and PCBs rank #5 on the ATSDR substance priority list (SPL). We investigated how well the new EPA method predicts measured bioaccumulation of PCBs in the food chain of two sites with PCB-contaminated sediment: 1) Ward Transformer Superfund Site at Crabtree Lake, NC USA and 2) Alcoa Site on Badin Lake, NC USA. We measured PCBs in water, zooplankton, and three different trophic levels of fish. We also measured PCBs in passive sampling devices (PSDs) designed to measure only the bioavailable PCBs in the water. We found that at the Ward Site the new EPA method was in good agreement with measured PCBs with some over-prediction by the EPA FCM model. At the Badin Lake Site, we found much greater variability and some under-prediction by the EPA FCM model that is perhaps due to less representative sampling at the Badin Lake Site. In both cases, variability and agreement was much better using PSD-derived bioavailable PCBs versus using PCB values directly measured in the water, owing to more representative long term exposure estimates using the PSDs versus grab sampling of water. Our work supports the use of the new EPA FCM method for PCBs and illustrates the importance of sampling representativeness to reduce uncertainty in predicting PCB accumulation in the aquatic food chain.

26 Effect of Black Carbon and Organism Growth Rates on Bioaccumulation of PCBs and Dioxin in Benthic Invertebrates

N. da Luz, K.J. Farley, Manhattan College / Civil and Environmental Engineering

Biota Sediment Accumulation Factors (BSAFs) are typically used as static descriptors for evaluating bioaccumulation of PCBs and dioxins in benthic invertebrates. However, large variations in BSAFs have been reported for laboratory bioaccumulation test results and for field-collected samples. Bioavailability of contaminants in sediments (e.g., as affected by the presence of black carbon) has been cited as a possible reason for these large variations. In addition to bioavailability, other factors such as the nutritional content of the sediment organic carbon, organism bioenergetics and feeding behavior are expected to play important roles. A "two box" bioaccumulation model was therefore developed to describe contaminant behavior in an invertebrate and its digestive tract. Ingestion (and egestion) of digestible organic carbon, non-digestible organic carbon and black carbon are considered separately. In the gut, digestible organic carbon is consumed and contaminant is allowed to re-partitioning between the various carbon phases. The model was applied to field data from New York-New Jersey Harbor and to laboratory bioaccumulation datasets for the Grasse River and Upper Hudson River. Results showed that calculated BSAFs will vary as a function of contaminant hydrophobicity, black carbon content and organism growth rates (which are linked to the availability of digestible organic carbon in sediments).

27 Modeling PCB Uptake in Fish after in-Situ Treatment of Sediment with Activated Carbon

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In-situ sediment amendment with sorbents such as activated carbon (AC) can effectively reduce the bioavailability of PCBs. However, there is limited experimental or modeling assessment of how bioavailability changes in sediments impact bioaccumulation in fish – the primary risk driver for exposure to humans and top predators in the aquatic ecosystem. In the present study we performed focused laboratory aquarium experiments and modeling to explore how PCB sorption in sediments impacted exposure pathways and bioaccumulation in fish. Porewater, overlying water, and fish tissue concentrations were measured in tanks containing either clean sand, PCB-impacted sediment or PCB-impacted sediment treated in the field with 2.8% d.w. AC. Treatment resulted in 96% reduction in total PCB concentration in porewater and 90% reduction in overlying water. Uptake of PCBs in worms exposed to treated sediment was less by 70% compared to worms exposed to untreated sediments. After 90 days of exposure, both fish showed reduced uptake of PCBs in tanks with sediment treated with AC (27 and 73% for cory catfish and mummichog, respectively). We show that by incorporating changes in porewater and overlying freely dissolved PCB concentrations in kinetic bioaccumulation models and by taking into account changes in food concentration it is possible to predict effectiveness of AC amendment in reducing PCB uptake in fish. In order to make these models more suitable for benthivorous fish that ingests sediments during feeding, assimilation efficiency of PCBs associated with sediment was independently measured. The modified model incorporating sediment ingestion was able to provide reasonable estimations of PCB uptake in the benthic feeding fish before and after treatment of the sediment with AC. The model was capable of predicting the dominant exposure pathways in the benthic and pelagic feeding fish as a result of their differences in biology. Furthermore, PCB exposure studies using passive sampling measurements were conducted that resulted in more accurate BCF-K_{OW} correlations for algae and zooplankton. The findings of the present study led to a refined bioaccumulation model that can predict uptake in the aquatic food chain, especially when bioavailability in sediments is altered.

28 Spatially-Explicit Bioaccumulation Modeling based on Passive Sampler Data: Result from Two Demonstration Sites

K. von Stackelberg, NEK Associates LTD / Environmental Health; P. Gschwend, MIT / Civil and Environmental Engineering

Food web models (FWMs) are used to predict fish tissue concentrations to support decision-making at contaminated sediment sites. These models currently rely on bulk sediment concentrations as exposure inputs, and typically average those (e.g., surface area weighted average), which can obscure contributions from specific locations. Here we report on two projects using polyethylene (PE) passive samplers to improve our ability to assess and remediate contaminated sediment sites. In the first, we use passive samplers to evaluate inputs of PCBs in the pore waters and overlying water column of the Lower Duwamish Waterway (LDW) site in Seattle, WA. Our ability to gauge the impacts of such contaminant inputs is examined using a probabilistic and spatially-explicit food web modeling approach. At the second site, we deploy PE samplers in both the water column and the sediment bed at Lake Cochituate in Wellesley, MA to obtain pore water and surface water concentrations of individual PCB congeners and use these as inputs to the FWM. We are doing this both at the area near the original spill and throughout the cleaner surroundings in two different seasons (summer/fall vs winter/spring). In addition to PE sampling, we are collecting sediments and biota at these times to 1) compare predictions to observed concentrations; 2) to contrast use of typical methods (e.g., use of bulk sediment data) with use of passive sampler data. In both cases, we hypothesize that a passive-sampler driven FWM will provide more accurate results than the sediment concentration-driven modeling, and that the spatially-explicit bioaccumulation model performs better than a deterministic, non-spatial approach. We report interim results for PE sampler data and resulting FWM predictions for the two sites. In the Duwamish, preliminary data suggests that sediment diffusive fluxes represent a small fraction of total observed PCBs in the water column. Ongoing studies of outfalls and other sources are providing insight into source contributions to the system. We duplicated the existing FWM and demonstrate resulting fish concentrations using more recent PE sampler data. For Lake Cochituate, we contrast the results of PE sampler data as compared to earlier bulk sediment concentrations and discuss implications for the FWM.

29 Quantifying Uncertainty in a Non-Steady State Bioaccumulation Model

A. McLeod, University of Windsor; D. Haffner, University of Windsor / GLIER

Although many persistent organic pollutants (POPs) have been banned from production and use, they remain a concern in the environment. Furthermore, many new, emerging chemicals of concern have the same toxic, persistent and bioaccumulative properties, thus there is a continuing need to quantify the hazard of these chemicals. The original structure of bioaccumulation models assumed steady state conditions with modifications allowing for trophic enrichment for chemicals that biomagnify where chemical fugacity increased with trophic level based on chemical assimilation efficiencies. These latter models were subsequently used to set water and sediment quality criteria for safe levels in fish as well to protect human health. However, there is a growing body of evidence suggesting that the assumption that chemical pollutants reach steady-state within an organism is not valid. Previously we demonstrated that a non-steady state (NSS) model improved predictions of chemical concentrations in a top predator. Despite these improvements, however, the model still demonstrates large amounts of model bias. Here, we use a NSS model and perform a sensitivity analyses to characterize the influence of different parameters on model accuracy. We observed that temperature had the strongest influence on model predictions across all $\log K_{OW}$s, followed by the chemical concentrations in food, and finally organism diet. Unfortunately, these parameters are also the most strongly influenced by anthropogenic climate change, invasive species, and food web collapses. However, by migrating from a steady-state bioaccumulation model to a NSS we are better able to predict the consequences of these multiple stressors in our changing world.

30 Assessment of sediment contaminant contribution to human health risk via bioaccumulation modeling

A.N. Parks, S.M. Bay, Southern California Coastal Water Research Project / Toxicology

Contaminated sediments pose not only a risk to the benthic community, but also have the potential to bioaccumulate and move through the food web, ultimately leading to human exposure via fish consumption. The State Water Resources Control Board (SWRCB) of California has developed a Human Health Assessment Sediment Quality Objectives Framework as a proposed amendment to the California Sediment Quality Objectives (SQO) Program. This new framework utilizes a food web bioaccumulation model in conjunction with contaminant exposure via fish consumption to determine the contribution of sediment contaminants to human health risk. This model utilizes site-specific water quality parameters (i.e., temperature, salinity, dissolved organic carbon, etc.) as well as measured sediment, water, and fish tissue contaminant concentrations in its calculations. The model predicts fish tissue concentrations based on measured sediment and water concentrations, which are then compared to the measured fish tissue concentrations to determine their relationship, or site linkage. These two parameters, chemical exposure and site linkage, are scored on a five- and four-point scale, respectively. These values are combined using a five-point scale to provide an overall assessment of the site: Unimpacted, Likely Unimpacted, Possibly Impacted, Likely Impacted, and Clearly Impacted. It is important to consider that both site linkage and chemical exposure are required to designate a site as impaired. In the case of high exposure but low linkage, the site of interest is not the main contributor of contamination suggesting the fish contamination may be due to off-site exposure. In the case of low exposure but high linkage, the human health risk is below the level of concern. This work provides a case study application of the model and framework as applied to a few bays and estuaries in California. Overall, this model and assessment framework are key tools needed to better understand and assess the impacts of contaminated coastal sediments on human health.

31 Food Web Models and Sediment Cleanup - Are We Asking Too Much?

D. Preziosi, R.A. Pastorok, Integral Consulting, Inc.

When cleanup is needed at Superfund sites, decision makers consider risk-based tools to assess how much cleanup is required in order to achieve protection of human health and ecosystems. When cleanup is needed for contaminants known to bioaccumulate and potentially biomagnify, or otherwise cause indirect and possibly cascading effects in food webs, use of mechanistic food web models have been promoted. The goal of this presentation is to present perspectives on common pitfalls in food web modelling and how they might be avoided, as well as lessons learned from good modeling practices garnered from other industrial sectors. Models for chemical uptake into food webs principally rely on mathematic expressions of first principles, including thermodynamics (e.g. fugacity). While first principles serve as the foundation, additional chemical, environmental state and species-specific inputs are imparted on the model. Alone and in combination the inputs can, often unnecessarily, complicate the model. To add further challenge, starting assumptions related to principal pathways of uptake, steady-state conditions, the presence of empirical relationship between media and tissue, the behavior and natural history of organisms, and the level of precision that can be reached by the model are often ignored or inadequately supported. Amidst high expectations and pressing timelines, what often follows are exercises in over-calibration to exert levels of precision and certainty that are at best optimistic, and at worst, can lead to billion dollar cleanups that will not achieve the desired risk reductions. We recommend that when food web models are used to support Superfund cleanups, that prioritization be placed on 1) first principle modeling approaches for ecology, not just physical chemistry; 2) collection of salient ecological data; 3) clear and defensible communication of the levels of precision and accuracy required for decision making relative to levels achievable through models; 4) systematic calibration through "evaludation"; and 5) use of good modeling practice consistent with existing international guidance (e.g., TRACE) for models used to support regulatory decision making. Without significant effort being devoted on such priorities, we urge that expectations be tempered and that caution be used when relying on food web models for Superfund cleanup.

32 Application of Food Web Models at Superfund Sites: Legal and Political Implications

K. O'Reilly, Exponent / Environmental and Earth Science

Even though there remains technical skepticism about their accuracy, food web models (FWMs) continue to be used at Superfund sites to forecast future fish tissue concentrations. The assumption that the results of modeling steady-state conditions can be used to predict the effect of sediment remediation remains controversial. This has not stopped such models from being critical components of decision-making at sites with remedial costs exceeding a billion dollars. In 2002, the U.S. Environmental Protection Agency (EPA) published "Principles for Managing Contaminated Sediment Risks at Hazardous Waste Sites," which addressed the use of FWMs at Superfund sites. The guidance called on site managers to clearly identify both the assumptions used in developing the models and the uncertainties in their output. Additionally, it recommended that the application of models at large or complex sites be peer reviewed, consistent with the Agency's Peer Review Handbook. In 2016, the Government

Accounting Office evaluated how the 2002 principles were being applied at Superfund sediment sites. Inconsistencies were noted across EPA regions concerning model assumptions, uncertainties, use of peer review, and stated confidence in future predictions. In response, EPA published a memorandum clarifying risk management recommendations. The agency acknowledged that technical uncertainties limit the ability of models to provide an accurate depiction of future conditions and that EPA documents have sometimes presented predictions of future sediment and fish concentrations "with a degree of certainty that fails to account for the inherent unknown accuracy of those predictions." Recent actions of the EPA Administrator indicate a move towards greater involvement of Headquarters to ensure consistency among Superfund sites. Differences between predicted concentrations and those measured in fish tissue as part the 2017 five-year review of the Hudson River PCB clean-up have initiated a controversy between state and federal regulators. This presentation will describe the evolving legal and political implications of the application food web models and hopefully start a discussion on how scientific advances can best be applied in managing risk at complex sediment sites.

Aquatic Toxicology and Ecology – Endocrine Disruptors

33 Evolution of the Medaka Extended One Generation Reproduction Test (MEOGRT)

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In response to various legislative mandates, the United States Environmental Protection Agency (USEPA) formed its Endocrine Disruptor Screening Program (EDSP), which in turn, implemented a tiered testing strategy to determine the potential of pesticides, commercial chemicals, and environmental contaminants to disrupt the endocrine system. The second tier of this testing strategy is intended to further characterize the effects on these pathways and to establish a dose-response relationship for adverse effects. The Medaka Extended One Generation Reproduction Test (MEOGRT) was developed by the USEPA's Office of Research and Development to fulfill the requirements of a Tier 2 test within the EDSP and published as the Office of Chemical Safety and Pollution Prevention Test Guideline 890.2200. In addition, a harmonized protocol was published by the Organisation for Economic Co-operation and Development (OECD) through collaboration between the USEPA and the Japanese Ministry of the Environment as OECD TG 240. The MEOGRT protocol was iteratively modified from a much longer research protocol based upon knowledge gained after successfully completing nine tests with variations in test protocols. After completion of these tests, both major modifications (e.g. increased replication) and minor modifications (e.g. choice of temperature) were incorporated into a final standardized MEOGRT protocol. The final protocol consists of continuously exposing parts of two generations of Japanese medaka (Oryzias latipes), starting with adult medaka (F0) exposed for 3 weeks and continuing into the F1 generation for 15 weeks post fertilization. Measurements of population-relevant parameters (i.e. survival, growth, and reproduction) are the primary emphasis of the MEOGRT protocol. However, these measurements are supplemented with measurements of liver vitellogenin levels, the number of papillae on the anal fin (an external sexual phenotype marker), and the histological evaluation of gonadal sex to help differentiate endocrine-mediated effects from systemic toxicity. These protocol iterations and the data associated with them provided valuable insights into nuances of the protocol. The various tests include exposure to 17β-estradiol, 4-t-octylphenol, o,p'-dichlorodiphenyltrichloroethane, 4-chloro-3-methylphenol, tamoxifen, 17β-trenbolone, vinclozolin, and prochloraz. The contents of this abstract neither constitute nor necessarily reflect USEPA policy.

34 Characterization of vitellogenin expression in male fathead minnow using current commercially available assays

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For decades, induction of the messenger RNA (mRNA) and yolk precursor protein, vitellogenin (VTG), in male fish has been utilized as an indicator of environmental exposure to estrogenic compounds. VTG protein has been measured by a variety of enzyme-linked immunosorbent assays (ELISAs). Recently, an ELISA targeting VTG from a variety of environmentally or regulatory relevant fish species was developed which detects VTG in serum, plasma, whole tissue homogenate and outer mucus coat (TECO Medical Group, Switzerland). Detection of VTG in mucus presents a non-invasive method of monitoring endocrine disruption. As the scientific community strives to reduce animal testing, this method could provide a reliable, minimally invasive sample for routine monitoring studies. The objective of this study was to characterize VTG expression in male fathead minnow (Pimephales promelas) mucus compared with more conventional measures in plasma and mRNA isolated from liver. VTG expression changes have frequently been studied in fathead minnows exposed to the well-characterized endocrine disrupting compound, 17α-ethinylestradiol (EE2). To assess the intensity and duration of changes in mucus VTG levels, fathead minnows were exposed to EE2 for 7 days with a subsequent depuration period of 14 days. The exposure was conducted in a flow-through system to maintain a consistent concentration of EE2 at a nominal EC50 dose of 2.5 ng/L and high dose of 10 ng/L as a positive control. Mucus, plasma and liver were sampled at regular intervals throughout the study. Gene expression increased after 2 days of exposure and disappeared by 4 days of depuration. Protein expression increased after 4 days of exposure and remained elevated in the high dose throughout the depuration period. The pattern of protein expression in mucus was the same as plasma indicating mucus can be used as a non-invasive sample for measuring VTG in fish. Sampling mucus allows researchers to collect more samples in less time during one sampling event without sacrificing animals. Additionally, the same population may be repeatedly sampled, which is potentially useful to monitor remediation efforts or for endangered species.

35 Adverse Effects of Triclosan on Testicular Development and Reproduction in Japanese Medaka at Environmentally Relevant Concentrations

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High concentrations of triclosan (TCS) have been reported in surface water and wild fish, however, it remains unclear whether TCS could induce gonadal intersex and cause reproductive abnormalities in fish at environmentally relevant concentrations. This study evaluated the adverse effects of TCS on gonadal development, reproductive inhibition and reproductive behavior by exposing male transgenic Japanese medaka (*Oryzias latipes*) from hatch for 100 days. TCS could significantly induce the incidence of gonadal intersex with the the lowest observable effective concentration (LOEC) of 117.9 ng/L and testicular development of male medaka with LOEC of 17.2 ng/L. The frequency of courtship in male medaka was significantly depressed with LOEC of 117.9 ng/L, and the hatching rates was inhibited at 17.2 ng/L of TCS. Since the LOECs were comparable to the concentrations in aquatic environment, TCS contamination would likely compromise the same reproductive abnormalities in wild fish. Male medaka exposure to mixture of TCS and E2β were also conducted to assess their potential synergistic effects. E2β at 2.4 ng/L enhanced an incidence and severity of intersex, retarded testicular development and reduced reproductive behavior induced by environmentally relevant concentrations of TCS. This study for the first time demonstrated the induction of intersex and reproductive effects of TCS and its synergistic effects combined with E2β at environmentally relevant concentrations.

36 Estrone and Temperature Interactions: Effects on the Predator-Prey Relationship in Freshwater Fish

V. Korn, J. Ward, H.L. Schoenfuss, St. Cloud State University / Aquatic Toxicology Laboratory

Contaminants of emerging concern (CECs), such as estrone (E1), have been studied extensively, however little is known about how temperature modulates these exposure effects and how predator-prey relationships are impacted. Previous studies indicate that changes may arise through behavioral and physiological changes in either predator or prey. To test the effects of E1 and temperature on predator-prey relationships, adult bluegills and larval fathead minnows were exposed to E1 (125, 625 ng/L) or an ethanol control for 30 days at four temperatures (15°C, 18°C, 21°C, 24°C). Larval predator evasion performance and feeding efficiency were tested on day 21. Temperature-dependent significant differences in body length, escape angle, and total escape response were observed upon analysis of predator evasion responses. On day 30, predation trials were performed using one adult sunfish and a mixed group of five control and five exposed (125 or 625ng/L) larvae. The concentration-dependent survival declined for exposed larvae (125ng/L: 49.2%; 625ng/L: 52.9%) when compared to the 74.2% of control minnows in the presence of the sunfish predator. The prey catching abilities of the sunfish were also affected, as control sunfish typically consumed a higher percentage of the larvae, potentially mitigating the predation effects on the minnows. This study provides evidence that minnow populations may suffer due to impaired predator evasion performance and provides information for environmental agencies evaluating ecological effects of exogenous estrogens and climate change.

37 Peroxisome Proliferator-Activated Receptors, Phthlates and Reproduction in the Zebrafish

G.J. Van Der Kraak, University of Guelph; A. Sritharan, University of Guelph / Integrative Biology

Peroxisome proliferator-activated receptors (PPARs) are nuclear hormone receptors that upon activation modify the expression of target genes involved in various physiological processes. PPARs play an important role in mammalian reproduction through direct effects on the ovary where they affect oocyte maturation, steroidogenesis and ovulation. Five different isoforms of PPARs (αA, αB, βA, βB and γ) have been identified in the zebrafish (*Danio rerio*) but unlike mammals, the presence of these isoforms in the ovary and their functions are largely unknown. Using real time quantitative PCR, we have shown that genes for all five isoforms of PPARs are present in the zebrafish ovary. In addition, we found that that the mRNA expression of certain PPARs (*pparαA*, *ppar βb* and *pparγ*) are higher in earlier follicular stages compared to more mature follicles suggesting a potential role of PPARs in ovarian follicular development. In separate experiments, injections of zebrafish with the known PPAR activators, di-2-ethyl-hexyl phthalate (DEHP; 50 and 500 mg kg^{-1}) and mono (2-ethylhexyl) phthalate (MEHP; 35.6 and 356 mg kg^{-1}) led to a significant dose dependent reduction in the numbers of eggs that were spawned. Fish treated with both phthalates had reduced mRNA expression of the nuclear progestin receptor (*npr*) in the ovary. Fish injected with MEHP also had reduced mRNA expression of a disintegrin and metalloproteinase with thrombospondin motifs1 (*ADAMTS -1*). *npr* and *ADAMTS*-1 are key regulators of the ovulation process in zebrafish. In summary, our results demonstrate the presence of PPARs in the zebrafish ovary and that the activation of these transcription factors may play an inhibitory role in ovarian follicle development and ovulation. Acknowledgements: Supported by a NSERC Discovery Grant to GVDK.

38 Global metabolomic profiling in larval zebrafish following exposure to the obesogenic DEHP (bis (2-ethylhexyl) phthalate)

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Obesity is a serious global public health challenge. Although poor nutrition and lack of exercise contribute to the disease, chemicals have also been shown to potentiate its etiology. A substantial body of evidence suggests that a subclass of endocrine- disrupting chemicals (EDCs), which interfere with endocrine signaling, can disrupt hormonally regulated metabolic processes, especially if exposure occurs during early development. These chemicals include plasticizers, such as bis(2-ethylhexyl) phthalate DEHP. This phthalate has been described as an obesogen, a chemical that disrupts lipid metabolism and produces metabolic changes. It has also been linked to increased risk of type 2 diabetes mellitus (DM) and cardiovascular disease (CVD). The aim of this study was to perform global metabolomics analysis on early stages of the zebrafish larvae, a model organism that has been used to study metabolic disorders. We exposed larvae to DEHP as there are reports that obesogens, including phthalates, are significantly higher in concentration in the urine of obese children, thus it is a sensitive life-stage for adverse effects of these chemicals. Larvae were exposed to either a DMSO solvent control (n=10) or 10 µM DEHP (n=10) for 96 hours with a 90% water change and census every 24 hours, starting at 6 hours post fertilization. There was no impact on mortality, morbidity, and hatch rate following exposure to 10 µM DEHP, which is in agreement with other studies reporting that DEHP is not overtly toxic to developing fish. To learn more about the molecular pathways perturbed by DEHP, fish were collected for metabolomics. Global metabolomics profiling was performed on a Thermo Q-Exactive Oribtrap MS with Dionex UHPLC. There were a total of 959 features detected from the positive mode and 174 features in the negative mode. Twenty-five metabolites were significantly different, of which 6 have been identified. These included D-raffinose, erythritol, 5-hydroxymethyl-2-furaldehyde, and N-acetylneuraminate. Relationships between these metabolites and obesity are currently being elucidated in conjunction with expression profiling for transcripts related to lipid synthesis, metabolism, and the storage including PPARs, leptin receptors, and adiponectin. This study improves mechanistic understanding of how plasticizers may impact vertebrate development as it relates to obesity in early development. This research was supported by Marie Sklodowska-Curie actions no.707241.

39 Unpublished: Effects of PCBs on expression of cancer and endocrine related genes in ninespine stickleback

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PCBs are known carcinogens and endocrine disruptors that can negatively impact reproductive, neurological and cellular function. St. Lawrence Island, located in the Bering Sea, was a strategic location for military bases during the Cold War. Two defense sites were built on St. Lawrence Island, including a radar surveillance station on the Northeast Cape (NEC). Despite remediation of the NEC formerly used defense (FUD) site, considerable levels of polychlorinated biphenyls (PCBs) remain in the local watershed. Contamination from the FUD site may pose a health risk to the 1,600 Yupik residents living on St. Lawrence Island. Studies have shown that blood serum concentrations of PCBs in St. Lawrence Island residents are six times higher than the national average. This study examines whether contaminant levels downstream of the NEC FUD site are significantly altering gene expression of cancer and endocrine related genes in ninespine stickleback (*Pungitius pungitius*). Ninespine stickleback are native to St. Lawrence Island and are useful as an indicator species because they share habitat and thus contaminant exposures with the local human population. Preliminary RNA-seq data show that 2,515 genes are differentially expressed in ninespine stickleback taken directly downstream of the FUD site relative to stickleback collected from upstream of the FUD site. Expression of BRCA1, BRCA2, BCCIP, BRIP1, DIO1, DIO2, and CYP1A genes are being analyzed in ninespine stickleback collected from sites both upstream and downstream of the NEC FUD site. Quantitative analysis of gene expression is determined through qRT-PCR. Results from this study are important for assessing genotoxicity of PCBs and optimizing methodologies for genetic analyses in ninespine stickleback. If results show that expression of target genes is significantly altered, it may indicate increased susceptibility to cancer and endocrine disruption for residents of St. Lawrence Island.

40 Detection of Human and Medaka Retinoic Acid Receptor Agonistic Activities in River Water in Japan

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Retinoic acid (RA) receptors (RARs) are nuclear receptors whose specific natural ligands are all-*trans* RA (atRA) and 9-*cis* RA (9cRA) derived from retinoid (vitamin A) precursors. RARs control aspects of vision, cell differentiation, immune response, and embryonic development in vertebrate. Due to the adverse effects by excess RAR signaling, the occurrence of RAR agonists in aquatic environments is a potential threat on human and aquatic animals. Therefore, this study aimed to elucidate the occurrence of the agonists on human and medaka RAR in river water environments in Japan. The RAR agonistic activities were investigated at 10 sampling stations in Sagami River in Japan during the period from October 2013 to September 2015. RAR agonistic activities of samples were measured by yeast two-hybrid assay targeting human and medaka RAR. Significant human agonistic activity ($p < 0.05$) were detected in most of all the samples irrespective of the sampling stations and seasons. Significant medaka RAR agonistic activities ($p < 0.05$) were also detected in most of all the samples, except some seasons. The results revealed that RAR agonists contamination were widespread in Sagami River investigated in this study. To characterize the RAR agonists, samples collected from several sampling stations during different periods were fractionated by reverse-phase HPLC. The fractions were collected every 2 min from 0 to 44 min (total of 22 fractions) and then each fraction was subjected to yeast two-hybrid assay. Irrespective of the sampling stations, seasons, and receptors, the fraction collected from 22 to 28 min commonly showed RAR agonistic activity. As the RAR agonistic activity was detected in 3 different fractions (i.e., 22-24, 24-26, and 26-28 min), multiple agonists which affect human and medaka RARs could occur in water samples in Sagami River. To identify the causative compounds, the concentrations of five typical RAs (atRA, 9cRA, 13cRA, 4-oxo-atRA and 4-oxo-13cRA) in the samples were analyzed by LC/MS. It revealed that the concentrations of 5 RAs in the samples were all below the detection limits. Thus, the occurrence of the other major human and medaka RAR agonists in Sagami River water was shown in the present study. Further investigation to identify the causative compound(s) is desired.

New Approaches to Long-Standing Challenges With Metals – TRV Development and Evaluating Effects in the Field

41 New approaches to long-standing challenges: Issues and solutions associated with TRV development

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Developing toxicology benchmarks for applications in risk assessment has been an evolving challenge for decades. Benchmarks are developed and applied to variety of media (tissue, dietary constituents, soil). Incomplete toxicity data sets, variation in response, differences in study conduct, and statistical error all contribute to variations in interpretation. For example, differences between species might be due to variation in toxicokinetics driven by environmentally weathered forms and differences in gastrointestinal physiologies. Physiological differences within a vertebrate class can drastically affect absorption; hence, can add substantially to a reduction in predictive capabilities of risk assessments. These issues are more complicated for metals which may be naturally occurring and, in some case, essential nutrients. Furthermore, focused use of negative data has yet to be elucidated in benchmark development. Here we present some of the challenges and sources for improvements in predictive toxicity benchmarks and provide some solutions for higher tiered risk assessments. In addition, we provide some suggestions for further research to help tease out predominant causes for variation.

42 Challenges in the assessment of soil metals exposure and risk within the CERCLA process

M.D. Sprenger, USEPA / OSRTI TIFSD ERT

It has been acknowledged that the chemical form of metals in soils is critical to both the bioavailabiliity and toxicity. A variety of methods have been used or proposed to determine the bioavailability and/or toxicity of metals in soils to wildlife; however, frequently both the technical experts and decision makers have been unsatisfied with the end results. A principle source of frustration is that interpretation and application of information is discounted within the risk assessment because of uncertainties and a desire to select a protective remedy, which translates into conservative decisions. Success in the application of the toxicological principles and advances in methodologies for assessing both the bioavailability and toxicology of metals in soils requires the risk assessment process to appropriately include and present information in the exposure assessment and hazard assessment. Consensus and or centralized toxicological information along with fully utilizing Problem Formulation could both streamline ecological risk assessments and increase confidence in the risk characterization. Advances in our understanding environmental toxicology need to be presented such that the science applied within the risk assessment demonstrably influences the risk characterization and thereby the decisions.

43 New Applications in EPA's ECOTOX Knowledgebase: Relative Potencies of Metals Across Chemical and Biological Species

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Toxicity of metals in field settings can vary widely among ionic chemicals and across biological receptors. Thus, a challenge often found in developing toxicity reference values (TRVs) for the risk assessment of metals is identifying the most appropriate metal and biological species combinations for risk characterization. In the problem formulation stage, investigators formulate the measurement and assessment endpoints most applicable to the environmental setting in question. A key step in this process is gathering toxicity data from literature to help identify relative sensitivities of biological species potentially found in trophic positions of the assessment's conceptual model as well as the potencies of metal species potentially identified for concern. The US Environmental Protection Agency's ECOTOX knowledgebase provides online access to curated literature-based toxicity effects data for organic and inorganic chemicals in ecological receptors. Recent enhancements to the ECOTOX knowledgebase have been completed to allow users to more easily review toxicity effects profiles for problem formulation purposes, and ultimately provide a source of toxicity data for developing dose-response models in TRV development. Reactive filtering of nearly 180,000 records from 19 groups of metals is now available using integrated data visualization capabilities to explore results of 406 chemicals, 4262 species, 24 effect groups with 1795 effect measurements. As a further example, ECOTOX includes 13 different chemicals associated with cadmium with effects data from 1659 species illustrating 24 effect groups and 698 effect measurements. The new and improved version of ECOTOX will allow risk assessors and researchers to visualize, identify and download curated effects data that best match exposure scenarios in conceptual models, help define the most appropriate assessment and measurement endpoints and be a source of toxicity data for TRV development. This abstract does not necessarily represent USEPA policy.

44 Dose-Response Toxicity Analysis of Mercury - Approach to Support Cleanup Decisions

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Ecological risks were evaluated at a contaminated tidal marsh system in the northeast United States consisting of 3,800 feet of tidal waterway and 17 acres of common reed (*P. australis*) dominated marsh. Mercury, polychlorinated biphenyls (PCBs), and other chemicals of potential concern (COPCs) are present in waterway and marsh sediments at the site and the broader region. The marsh and waterways are a valuable resource actively used by a variety of wildlife. An assessment approach was designed to characterize the nature, magnitude, and likelihood of potential adverse effects due to COPC exposure. The baseline ecological risk assessment (BERA) used deterministic and probabilistic exposure modeling and dose-response toxicity analysis to inform cleanup decisions with technically defensible data evaluations that met the EPA risk assessment framework. Indirect food web exposures for wildlife were estimated by modeling chemical-specific concentrations in dietary components using combinations of site-specific and literature-based bioaccumulation models and/or measured tissue concentrations. Given varying availability of data for many exposure pathways, a series of deterministic exposure estimates were produced to focus probabilistic estimates on key pathways. Detailed review of avian toxicological data for mercury was performed, including developing dose response relationships. Effect thresholds were established as ED20s (dose resulting in a 20 percent effect). Probabilistic models of exposure were run and were compared to ED20s to quantify likelihood and magnitude of adverse effects. Deterministic models indicated low to medium level risk within the marsh but did not inform likelihood of occurrence. However, probabilistic model results compared to the deterministic ED20s showed a low probability of adverse effects within the marsh habitat. Without a probabilistic risk evaluation, site management decisions could be based on perceived risk with low likelihood of occurrence. The use of probabilistic risk assessment, combined with dose-response analysis of toxicity data to develop risk thresholds, will result in a more informed risk management decision. The more informed decision will likely be more cost effective and may avoid active remediation that would have caused an unnecessary temporary loss of ecosystem function.

45 Development of Metals Toxicity Reference Values for Birds and Mammals Using Benchmark Dose Modeling

B.A. Bergquist, Windward Environmental LLC; A. Fairbrother, Exponent, Inc. / EcoSciences; N. Judd, Windward Environmental, LLC

We have developed and implemented an approach for wildlife toxicity reference value (TRV) development that includes benchmark dose modeling in support of an ongoing ecological risk assessment involving numerous metals of potential concern. TRVs were developed for five metals (cadmium, copper, lead, manganese, and zinc) for both birds and mammals, based on growth, reproduction, and survival endpoints. Using toxicity data sets from studies that met *a priori* established acceptability rules, we modeled dose-response functions and calculated effect doses corresponding to a 20% effect level (i.e., ED_{20}). ED_x-based TRVs are preferred over no observed adverse effect levels (NOAELs) and lowest observed adverse effect levels (LOAELs) because they are more accurate predictors of risk at a particular response level. We evaluated two EPA software programs for development of dose-response functions: Benchmark Dose Software (BMDS) and Toxicity Relationship Analysis Program (TRAP). Data from different studies with comparable datasets (i.e., same species, same chemical form, same exposure duration, etc.) were pooled prior to modeling. A meaningful selection among the multitude of BMDS models required more robust data than were provided in the toxicity studies in most cases. TRAP was the preferred modeling method because it required fewer inputs and generated a smaller, but still sufficient, array of generic models. Even still, many of the available data sets did not meet established minimum data requirements for modeling ED_{20}s. If an ED_{20} could not be derived from a study's toxicity dataset, a lowest observed adverse effect level (LOAEL) was derived. For each metal and endpoint combination, the lowest ED_{20} or LOAEL from the available studies was selected as the TRV. Almost half of the TRVs were based on modeled ED_{20}s, and the remainder were based on LOAELs. The ability to derive and use ED_{20}-based TRVs is expected to provide a more accurate estimate of acceptable risk than if only LOAELs were used in the risk assessment, but the ability to develop ED_{20}s is limited based on available toxicity data.

46 Metal soil threshold calculator tool: Derivation of ecological metal soil quality standards for different scenarios and protection goals

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During the last 2 decades, intensive research has been performed to improve the risk assessment of metals in soil and numerous chronic metal toxicity data were generated for various terrestrial species and microbial functions in different soil types. Models were developed for correction for differences in bioavailability among soils and for differences between laboratory and field conditions. For many metals, the large amount of chronic toxicity data for different species and functions allow the application of the species sensitivity distribution approach (SSD) to derive soil quality standards. This work was mainly triggered by the European legislation on chemical management (REACH) and the data were therefore primarily used to derive predicted no effect concentrations (PNEC) for prospective risk assessment. To facilitate a more flexible derivation of ecological quality standards for metals in soil for different protection goals (e.g. remediation thresholds), jurisdictions, regions or sites, while still making maximal use of the wealth of data and models already available, a metal soil threshold calculator tool has been developed. This freely available spreadsheet reports almost 1200 reliable chronic toxicity data for the direct effects of the metals Cd, Co, Cu, Pb, Mo, Ni and Zn to soil organisms (plants, invertebrates and microbial processes) and calculates ecotoxicological threshold concentrations expressed as (pseudo-) total (i.e. aqua-regia extractable) metal concentrations in soil (mg/kg dry weight). All metals covered have sufficient chronic toxicity data allowing the derivation of an SSD. The soils used for ecotoxicity testing cover for each metal a wide range of soil properties, making the results representative for most regions in the world. Several options are included to allow calculation of metal soil threshold concentrations for various goals (e.g. risk assessment or setting of remediation thresholds for different land uses): selection of organism groups or species to be considered, selection of effect levels from the original dose-response curves (ECx), selection of protection level (probability level in SSD), bioavailability models to be included etc. The advantages of this approach are the maximal use of available toxicity data and bioavailability corrections and the enhanced transparency in the derivation of ecological quality standards for metals in soil for different goals and different scenarios.

47 When site specific TRVs are not an option - Improving consistency of risk assessments for the Canadian Federal Contaminated Sites Action Plan

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Ecological risks assessments for contaminated sites utilize either published toxicity reference values (TRVs) or TRVs that are specifically developed for a particular site. The Canadian Federal Contaminated Sites Action Plan (FCSAP) encourages the development of site-specific TRVs and has developed guidance on scientifically defensible methods for TRV development applicable to metals and other contaminants. However, for many federally managed sites, developing site-specific TRVs is beyond the scope of the remediation or risk management project. Instead, risk assessors often choose an existing published TRV to assess the risk to ecological receptors. Published TRVs vary widely in their numeric values, derivation methods, uncertainties, and in the level of protection they provide. Subsequently, the outcome of the risk assessment can vary depending on which published TRV is applied. This leads to inconsistencies when similar sites are assessed by different practitioners, organizations or regions. To streamline risk assessments where development of site-specific TRVs is not feasible, FCSAP has developed an approach to evaluate published TRVs and identify those which are best suited for use as standard default TRVs. This approach ranks available TRVs for metals and other contaminants based on how well they align with existing FCSAP guidance. The evaluation is based on a set of ten criteria, including whether the TRV is based on dose-response data versus unbounded or bounded point estimate data. Other evaluation criteria include use of uncertainty factors, allometric scaling, biological endpoints, test species, and protection levels. FCSAP has applied these criteria to select recommended default wildlife TRVs for fourteen metals and nine organic compounds. The selection rationale provides a suitability ranking as well as a detailed description of the merits, limitations, and uncertainties of commonly used published TRVs on a chemical-by-chemical basis. Only three of the evaluated metal TRVs were generally consistent with FCSAP guidance and are recommended as default standard TRVs for FCSAP sites without limitations. Other TRVs diverge from FCSAP guidance to varying degrees and are ranked accordingly. The ranking and detailed evaluation can assist managers and practitioners in prioritizing where further site-specific TRV development work may be warranted at federal contaminated sites.

48 A Proposed Framework for Incorporating Dietborne Metal Toxicity Thresholds into Aquatic Life Risk Assessments

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The potential for dietborne metals toxicity to aquatic life has received increased attention over the last 15 years. However, the dietborne metals pathway is typically either not evaluated or inconsistently evaluated in aquatic life risk assessments. We will present a framework for incorporating dietborne metals toxicity into aquatic life risk assessments, including considerations during the problem formulation and exposure, effects, and risk characterizations. An important component of the problem formulation is developing the analysis plan that describes how the dietborne metals line of evidence may be evaluated relative to other lines of evidence, such as metal concentrations in water, sediment, and tissue. Important considerations in the exposure characterization include the types of food chain data that are needed for defining dietborne exposures to the aquatic life receptors of interest and the relative benefits in evaluating concentration-based versus dose-based dietborne metals exposures. In defining dietborne toxicity thresholds in the effects characterization, an important consideration is whether the diets in the tests used to derive the toxicity thresholds are based on biological or formulated diets. Recommendations for then integrating the dietborne metals evaluation with other lines of evidence in the risk characterization will be presented, as well as how dietborne metal mixture studies may be used to support interpretation of the individual metal risk conclusions. Finally, we will summarize uncertainties in evaluating dietborne metals risks and how these uncertainties may be reduced by future studies.

Recent Developments and Current Issues in Bioaccumulation Assessment

49 Investigating the Effect of Fish Size on Bioaccumulation Using an Integrated Bioenergetics-Bioaccumulation Model

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Because dietary uptake of fish is often a major vector of human exposure to persistent organic pollutants (POPs), much effort is directed towards a quantitative understanding of fish bioaccumulation with the help of mechanistic models. Such models require the input of the growth, feeding and respiration rates of a fish. However, often little consideration is given to the interdependency of these physiological parameters. Here, we calculate the bioaccumulation factor (BAF) of hypothetical POPs, with log K_{OW} values ranging from 4.5 to 8.5, in lake trout (*Salvelinus namaycush*), with a food web bioaccumulation model that uses bioenergetic equations to ensure that the physiological parameters applied to a species are internally consistent (i.e., energetically balanced). Empirical growth rates and diets for lake trout in six Canadian lakes (Great Slave Lake, Lake Ontario, Source Lake, Happy Isle Lake, Lake Opeongo and Lake Memphremagog) are used to determine feeding rates. Respiration rates were derived based on the routine metabolic rates and the population specific activity coefficients (multipliers). When comparing differently sized lake trout within a lake, larger fish tend to have the highest BAF, because they allocate relatively less energy towards growth than smaller fish. When comparing fish from different lakes, diet composition and activity become important in determining BAF in addition to the amount of total energy allocated to growth. Specifically, fast growing Lake Ontario lake trout, feeding on slow growing alewife, have higher BAFs; while slower growing small lake trout in Happy Isle and Source Lakes have low BAFs because they feed on invertebrates, which are low in the food chain.

50 Testing in vitro to in vivo extrapolation approaches for assessing biotransformation rates and bioaccumulation factors in fish

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Recent studies demonstrate the utility of in vitro to in vivo extrapolation (IVIVE) approaches for predicting the impact of biotransformation on chemical bioaccumulation in fish. However, it remains important to test the domain of applicability of these IVIVE approaches before such methods are incorporated into hazard and risk assessment frameworks. A major limitation of testing current IVIVE approaches is the lack of empirical in vivo biotransformation data to directly evaluate extrapolated in vitro predictions. Here we present the results of an in vivo dietary bioaccumulation test (modified OECD 305) performed for two organic sunscreen agents (ultraviolet filters; UVFs), ethylhexyl trimethoxycinnamate (EHMC) and octocrylene (log Kow of 5.8 and 6.9, respectively). Rainbow trout were exposed to three dietary concentrations of each compound to investigate the relationship between dietary exposure concentration and observed accumulation and elimination. Non-biotransformed reference compounds were also incorporated to provide a basis for measuring in vivo biotransformation rate constants (k_{MET}). This study showed that both UVFs were significantly biotransformed, providing mean k_{MET} values of 0.25 and 0.09 d^{-1} for EHMC and octocrylene, respectively. UVF k_{MET} values did not differ between dietary exposure concentrations, indicating no apparent concentration dependence of in vivo biotransformation rates in fish. Measured UVFs concentrations in the fish may not have been sufficiently high enough to reach concentrations that saturate biotransformation enzymes. The EHMC k_{MET} value corresponded well to k_{MET} values estimated from in vitro biotransformation data measured in hepatic bioassays. However the measured k_{MET} for octocrylene was about 2-fold higher than the k_{MET} estimated using in vitro hepatic biotransformation data. We propose this deviation is due to significant in vitro intestinal biotransformation that has been observed for octocrylene. The results of this study suggest a need to include in vitro methods for intestinal biotransformation to further improve the application of the IVIVE approach for bioassessment assessment.

51 The Bioaccumulation Assessment Tool: Overview and Case Study Application

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World-wide, in various regulatory contexts, chemicals are being evaluated for their potential hazard, exposure and risk to ecological receptors and humans. Thousands of chemicals are being screened for their Persistence, Bioaccumulation and Toxicity (PBT). Bioaccumulation can be characterized using data collected in the laboratory and field and using in silico approaches. Lab-based lines of evidence (LOE) for assessing bioaccumulation include the octanol-water partition coefficient (K_{OW}), the bioconcentration factor (BCF) and the biomagnification factor (BMF). In vitro biotransformation rate data (e.g., from S9 and hepatocyte assays) can also be considered through in vitro to in vivo extrapolation (IVIVE) methods. Field-based LOE include the BMF, the bioaccumulation factor (BAF), and the trophic magnification factor (TMF). In silico LOE include quantitative structure-activity relationships (QSARs) for the BCF and the biotransformation rate constant (k_M) and mass balance bioaccumulation models. The Bioaccumulation Assessment Tool (BAT) aims to consolidate relevant measured and modelled data as listed above. The BAT is a user-friendly, organizational framework and computational tool in the form of an Excel/VBA spreadsheet that integrates various LOE using a quantitative weight of evidence (QWOE) approach to guide bioaccumulation assessment. The various LOE are treated as “Input” to the BAT. The BAT uses Data Evaluation Templates (DETs) to characterize the data confidence (quality) from the various LOE based on best scientific practices (e.g. OECD 305 test guidelines) to address uncertainty. Primary “Output” from the BAT includes a comparison of these LOE against user-defined criteria/thresholds to assign a B-designation for each and consensus in classifications is quantified. Using a QWOE approach to integrate this information, an overall bioaccumulation classification is provided. The BAT seeks to facilitate transparent and consistent bioaccumulation assessment and provide strategic guidance for integrated (tiered) testing to address remaining uncertainty in the available data, as warranted. The development of the BAT includes multi-stakeholder participation and the BAT will be freely available. A case study application of the BAT is presented to demonstrate the work flow and assessment outputs.

52 Comparison of Bioaccumulation in Three Freshwater Species in an Inter-Laboratory Assessment of a Laboratory In-Vivo Bioaccumulation Test Method

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Standardized bioaccumulation testing of aquatic organisms is essential to understanding the impact of historical contamination on the quality of water and sediment. Input of contaminants into aquatic ecosystems has been reduced through the regulation of discharges, however historical contamination continues to impact the quality of water and sediment. In addition to the physicochemical characterization of sediment, toxicity testing, and benthic surveys, an assessment of the bioaccumulation of contaminants is now part of the decision-making framework for contaminated sediments in Ontario and elsewhere. It is not always practical to collect enough sediment dwelling organisms of a single species for tissue analysis in the field and due to the uncertainty associated with the ability of various bioaccumulation metrics to assess bioaccumulation potential it is of value to have a reliable and repeatable laboratory in-vivo bioaccumulation test method. The University of Guelph and the Ontario Ministry of the Environment and Climate Change (MOECCC) partnered to build on the existing USEPA Lumbriculus variegatus test methodology and developed a standardized method which also included an insect (the freshwater burrowing mayfly Hexagenia spp.) and vertebrate (juvenile fathead minnow). This method has been used to better understand species-specific differences in bioaccumulation and has been used regularly by the MOECC in sediment risk assessments. An interlaboratory comparison was conducted to assess the precision of this method. Three field collected non-toxic sediments contaminated with bioaccumulative compounds were used in this study. Sediment contaminated with arsenic was tested with Hexagenia spp., polycyclic aromatic hydrocarbon (PAH) contaminated sediment was assessed using L. variegatus, and sediment with high levels of polychlorinated biphenyls (PCBs) was tested with fathead minnows. Control and test sediment were sub-sampled and sent to six (*Hexagenia* spp.) or seven (*Lumbriculus variegatus* and *Pimephales promelas*) laboratories to perform the bioaccumulation test. Survival, growth and tissue accumulation of the contaminant of concern will be presented and method precision, challenges and opportunities for method refinement will be discussed. The authors will discuss the challenges associated with obtaining International Standards Organization (ISO) accreditation for this method.

53 Assessment of Bioaccumulation Potential in USEPA TSCA New Chemicals Assessments

D. Tobias, M. Card, D. Lynch, K. Eisenreich, USEPA / Office of Pollution Prevention and Toxics

The U.S. Environmental Protection Agency Office of Pollution Prevention and Toxics, Risk Assessment Division (OPPT/RAD) assesses over a thousand new chemicals per year under the Toxic Substances Control Act (TSCA). Under TSCA, prior to manufacture or importation into the United States, new chemicals must undergo review to evaluate human and ecological risk. Review includes the assessment of environmental persistence and bioaccumulation. Measured data on bioaccumulation is rarely received with new chemical submission, thus, OPPT/RAD scientists must use models and analog data to inform bioaccumulation assessments. To assess bioaccumulation, OPPT/RAD scientists mainly use the BCFBAF model within EPISuite to evaluate new chemical submissions, along with analog data from sources like Japan’s J-CHECK database and a general knowledge of literature data for chemical classes. This presentation will describe how model and analog approaches help describe the bioaccumulation potential and connect with the new chemical category document on persistent, bioaccumulative, and toxic (PBT) chemicals. The BCFBAF model is based on publically available data and OPPT/RAD is looking to improve both the performance of the model and the understanding of which components of the BCFBAF or other bioaccumulation models are most relevant for different chemical classes. The BCFBAF model includes a QSAR to estimate fish metabolism and additional in vitro fish metabolism data would improve the accuracy of its half-life predictions for the range of chemicals reviewed by OPPT/RAD. Bioaccumulation assessment of an individual chemical can also be improved by directly entering a measured in-vitro metabolism half-life into bioaccumulation models. There are opportunities for additional models to contribute to bioaccumulation assessments, if they conform to limitations required for federal regulatory activities and TSCA. The requirements for models are based on the need for transparency in model methodology, availability to stakeholders, and protection of confidential business information. The views expressed in this abstract are those of the authors and do not represent Agency policy or endorsement.

54 Uptake and biotransformation of sediment-associated cyclic volatile methylsiloxanes: Comparing a freshwater oligochaete and an estuarine polychaete

H. Selck, R. Windfeld, Roskilde University / Science and Environment

Chemical regulatory legislation of organic contaminants is generally based on an assessment of the chemical potential to persist (P) in the environment, bioaccumulate (B) in biota, and possess potential toxicity

(T). Applying standardized exposure setups (i.e., water-only exposure) as historically has been employed in environmental risk assessment, may underestimate bioaccumulation of hydrophobic organic contaminants (HOCs) in sediment-dwelling organisms because: 1) HOCs often accumulate in sediments to concentrations greatly exceeding the concentration in the overlying water; and 2) a number of papers illustrate that sediment-associated HOCs are available for uptake in benthic organisms. Alternatively, benthic invertebrates may be able to metabolize organic contaminants (i.e., biotransform), thus reducing their body burden. However, available information on the biotransformation capacity of benthic organisms is very limited. We conducted a number of experiments examining uptake and biotransformation of sediment-associated cyclic volatile methylsiloxanes (i.e., D4 and D5) in two deposit-feeding worms, namely, the estuarine polychaete, *Capitella teleta* and the freshwater oligochaete, *Tubifex tubifex*. This presentation will provide examples of how biotransformation capacity varies among the two benthic deposit feeders, and how biotransformation may reduce body burden and facilitate the removal of sediment-associated siloxanes. Including these factors in a hazard or risk assessment are likely to impact PBT assignment and categorization, and exclusion of benthic organism behaviour may add compound uncertainty to predictions of bioaccumulation and trophic transfer.

55 A Reference Database for Bioaccumulation of Organic Chemicals in Worm

D. Kuo, City University of Hong Kong / Architecture and Civil Engineering

Worms provide critical ecological functions in benthic and terrestrial environments by maintaining good pore structure, promoting nutrient cycling, and raising productivity. Their continuous interaction and processing of deposits/particles making them an ideal indicator species for pollution in soils and sediments. Predicting the bioaccumulation potential of organic chemicals in worms, however, remains a major challenge due to the lack of a high quality database. This study attempts to construct a comprehensive worm bioaccumulation database that cover both soil and sediment environments and to identify the overall status and research needs. Worm bioaccumulation studies were reviewed and processed for bioaccumulation measurements (i.e., BCF and BSAF), kinetics of contributing processes, and metric nature. In addition, experimental conditions, exposure medium characteristics, and biological properties are also documented and reviewed. Approximately 3500 bioaccumulation data entries covering 300 non-ionic and ionizable organic compounds were compiled from over 150 studies. BSAF measurements account for over 95% of the data, indicating the significance of soil/sediment exposure in these studies. These measurements are equally divided between field exposure and laboratory uptake studies. Non-ionic organic chemicals, with a strong focus on legacy contaminants, account for over 85% of the data entries; data entries for ionizable organic compounds are relatively minor (≤ 5%). Over 40 oligochaete and polychaete were documented. Oligochaete contribute for two-third of all laboratory bioaccumulation data; polychaeta studies, however, mostly involve field animals. A number of research needs for future bioaccumulation studies have been identified. Comprehensive kinetic characterization bioaccumulation process is needed as basic uptake /depuration kinetics are only available in < 15% of the entries. Biotransformation kinetics is largely absent except for limited legacy contaminants. While soil/sediment organic carbon is frequently reported, black carbon – which can significantly modify the bioavailability of organic contaminants – is rarely reported (< 5%). Basic biological properties such as body weight and lipid content are only reported in half of the entries. Implications of these limitation toward mechanistic bioaccumulation modeling, risk assessment, and chemical management will be discussed.

56 Mercury Bioaccumulation in Freshwater Fish Is Inversely Related to Environmental Selenium

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A recent study assessed the mercury (Hg) and selenium (Se) concentrations in >14,000 ocean and freshwater fish has found the majority of ocean fish contain far more Se than Hg, and will therefore prevent than cause Hg toxicity. However, because the Hg contents of freshwater fish are inversely related to environmental Se availability, poor Se accessibility can accentuate both the amount of Hg exposure associated with fish consumption as well as the associated risks. For this reason, the effects of methylmercury (MeHg) exposures from fresh water fish consumption require a better-informed approach to risk assessments. In all vertebrate tissues, MeHg initially binds to thiols, but since substrate thiols are directly acted upon by certain selenoenzymes, these species become “suicide substrates” that actively deliver MeHg into the active sites of Se-dependent enzymes in the proper orientation to accomplish its transfer from the substrate thiol to the enzyme selenol. These MeHg-Se conjugates degrade to form mercury selenide (HgSe), an insoluble form that cannot be absorbed during digestion. This retires Hg from the biogeochemical cycle, and diminishes the amount available in tissues of predators or human consumers. Because the soils of many North American watersheds are Se-rich, fresh water fish from most regions contain far more Se than MeHg. However, fish from certain regions of North American and many other regions of the world have poor Se availability, either due to low abundance in the geological parent rock material, or due to poor Se bioavailability due to low pH of the watershed’s soil or waters. Poor Se availability in freshwater bodies was found to greatly increase MeHg bioaccumulation in fish. In the absence of sufficient Se, freshwater fish bioaccumulate more MeHg than fish from Se-rich watersheds, and their poor Se contents greatly increase the risks associated with these MeHg exposures. Therefore, these aspects of MeHg associated risks urgently require further study, especially in regions of the world which are known to be Se-poor. The risks associated with eating fish from such watersheds will need to be established on a case by case basis. In summary, MeHg risks cannot be accurately evaluated using methods that only consider MeHg exposures. The Se-Health Benefit Value (HBV) is intended to enable rapid identification of areas at accentuated risk and provide reliably accurate assessments of MeHg-exposure related health hazards.

Development of Microbial Environmental Resistance

57 Resistance, adaptation, acclimation? Which one is it? When is it really resistance?

M.C. Capdevielle, S. Shah, M. Johansson, Colgate-Palmolive Company; G.I. Scott, University of South Carolina / Env. Health Sciences

The World Health Organization defines antimicrobial resistance (AMR) as the ability of a microorganism (like bacteria, viruses, and some parasites) to stop/delay/inhibit/render inactive an antimicrobial (such as antibiotics, antivirals and antimalarials) from working against it. As a result, standard treatments become ineffective, infections persist and may spread to others. Often mentioned with resistance, is, adaptation. Adaptation can occur in two ways, inherent, often referred to as intrinsic or acquired. Recently there is much discussion on environmental resistance and there is varying perspective on what criteria is required to determine if environmental resistance is being triggered directly by a chemical or compound or is there an indirect pathway by causing molecular changes in gene expression resulting in development of plasmids which may be exchanged within a bacterial species and/or among different bacterial species. When questioning if an antibiotic or antimicrobial can cause the development of resistant strains of bacteria, a number of criteria should be considered. Are results seen only in vitro in the laboratory? Is the emergence of resistance induced/selected by a compound related to the genetic control on the resistance gene(s) present on chromosomal and genetic mobile elements (plasmids) in vitro? How does this compare to what has been observed in the environment? How does exposure concentration and/or persistence influence development of resistance? Mechanisms are often associated with resistance development are often linked to changes in permeability or in efflux. It is also possible that target biomolecules are modified by post-translational modifications. There are varied opinions on what defines bacterial resistance and what would confirm if resistance development has occurred. This presentation is a primer, for the presentations to follow in this session and will focus on these different and complex issues related to environmental antibiotic resistance.

58 Biofilms as a natural antibiotic-resistant bastion for bacteria

A. Decho, University of South Carolina / Environ. Health Sciences

Biofilms are attached forms of bacteria where cells are surrounded by a protective matrix of extracellular polymeric secretions (EPS). Biofilms are the predominant state for most bacteria, including pathogens, in natural environments and during human infections, and therefore pose a crucial link between the local environment and health. This talk will examine how biofilms are inherently designed to foster the development of resistant forms of bacteria, and coordinate resistance mechanisms to antibiotics at several different levels. The EPS matrix is the primary emergent property of a biofilm. Its presence facilitates cell-cell chemical communication (i.e. quorum sensing), which coordinates group activities to defend against antibiotic intrusion. The matrix localizes extracellular enzymes that degrade incoming antibiotics and lipid vesicles (containing the enzymes), which in turn, creates destructive minefields for antibiotics diffusing toward cells. The EPS matrix also contains (inactive) persister cells, which are relatively immune to effects of antibiotics, enhances gene (plasmid) exchange, slows diffusion (to reduce antibiotic penetration), and even permits survival of cells and enzymes during desiccation. Examples of these processes will be provided. Together, these capabilities provide biofilm cells with an organized, highly adaptable and resistant bastion against antibiotics and other antimicrobial compounds. This adaptable biome facilitates the persistence of pathogens, as well as other pathogen-supporting bacteria, in unexpected locations in both natural and engineered environments.

59 Antibiotic resistance genes are emerging contaminants in surface waterways impacted by agricultural practices

R. Beattie, M. Walsh, K. Hristova, Marquette University / Biological Sciences

Increased anthropogenic activity has significantly altered the presence of antibiotic resistance genes (ARGs) in the environment. Although ARGs occur naturally, antibiotic use in livestock farming is implicated as a source of increased dissemination of ARGs in the environment primarily via manure runoff. As connections between environmental and clinical ARGs continue to emerge, a better understanding of the impact of field crop manure application on the abundance and spread of ARGs is necessary to mitigate the rising public health concern of antibiotic resistance. Kewaunee County, Wisconsin is an agricultural region dominated by concentrated animal feeding dairy operations and manure-fertilized cropland, two practices that impact soil and water quality. Seasonal, locational, and environmental contamination induced fluctuations of ARGs are expected due to manure application and runoff. Molecular approaches were utilized to assess ARGs and environmental variables in Kewaunee River water and sediment samples collected seasonally. Four ARGs, one integron-integrase gene, and 16S rRNA gene were quantified by qPCR in surface water and sediment samples (n=20), with detection of all genes in both sample types at all locations. All ARGs were present in higher copy numbers in sediment samples. Bovine bacterial fecal markers in water were positively correlated with ARGs in both water and sediment. Rainfall events correlated with increase copy numbers of all measured genes. Surprisingly, ARGs were found to be negatively correlated with proximity to dairy operations; however, sampling locations further downstream (and therefore subject to accumulation effects) were found to contain the highest copy numbers of ARGs. Additionally, trace amounts of antibiotics tetracycline and sulfamethazine were detected in surface waters. Principle Component Analysis results indicate that phosphorus (a primary component of manure fertilizer) explains a significant amount of variation in the ARG abundance. These data implicate sediment as a reservoir of ARGs and are indicative of long-term environmental contamination. Additionally, trace amounts of antibiotics in the environment may serve as a selective factor for maintaining a diverse pool of ARGs. Together, these results implicate manure spreading practices in the dissemination and abundance of ARGs, necessitating the need for mitigation of antibiotic resistance spread by changing current manure application practices.

60 Risk assessment of antibiotic resistance and related genes in human impacted environments

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The origins of antibiotic resistance in the environment is relevant to human health because of the increasing importance of zoonotic diseases as well as the need for predicting emerging resistant pathogens. Antibiotics are used in diverse settings for food production. Domestic animals are treated with antibiotics for both curing disease and promoting growth. Moreover, aquaculture relies on antibiotics to manage infectious disease. Wastewater treatment plants receive sewage from various sources, including hospitals and households which are both important sources of antibiotics and their residues, and antibiotic resistant bacteria. Risk assessment of antibiotic resistance is complicated. It should include at least quantitative information of the gene, sequence of the gene, host cell of the gene and genetic environment of the gene (e.g. presence in mobile DNA element). We have used the combination of different methods for obtaining that information: Parallel quantitative PCR array for high throughput quantification (1), epicPCR(2) for host information and Inverse-PCR(3) for analysis of the genetic environment. Inverse-PCR and epicPCR combined with DNA sequencing resolve also the sequence of the resistance gene. Samples were collected from different locations in Finland: manure from cattle and pig farms, soil that received the manure as fertilizer, sediments from aquaculture farms and effluent, influent and activated sludge from waste water treatment plant. Our results demonstrate that human activities results to the increase to the abundance of antibiotic resistance genes. In many cases the genes are located in mobile genetic elements with increases the probability of transfer of the them between bacterial species. The host range information obtained by epicPCR revealed wide diversity on the host range of the antibiotic reistance genes in different environments. Our results can be used for the development ecotoxicological risk analysis for antibiotic resistance.

61 Relationship between Total Silver Concentration and Sil Silver Resistance Genes in Domestic Wastewater Treatment Plants

C. Gwin, Bucknell University / Civil & Environmental Engineering; C. Gunsch, Duke University / Civil and Environmental Engineering

The use of silver nanoparticles in consumer products has been increasing over the past decade due to their antibacterial activity. In 2013 there were already 383 products containing nanosilver, and this number continues to grow. Due to this increase, the evolution and fate of silver resistant wastewater bacteria is an important area of research as the subsequent influx of silver nanoparticles and ionic silver into wastewater is likely to produce additional silver resistant bacteria. TaqMan qPCR assays for four *sil* silver resistance genes, *silC*, *silP*, *silR*, and *silS*, were developed to quantify the prevalence of these genes in domestic wastewater treatment plants (WWTP). Fifty ml of activated sludge from seventeen WWTPs located across NC, PA, OH, WA, and OR were collected and used to isolate DNA, while the silver concentration was determined using microwave digestion followed by ICP-MS analysis, and Chemical Oxygen Demand (COD) was determined using the COD HR kit. The Spearman Rank Correlation was performed in R. The WWTP parameters examined included size, COD, and total silver concentrations, as well as gene copy numbers for *16S*, individual *sil* genes (*silC*, *silP*, *silR*, *silS*), and total *sil* gene copy numbers. The size of the WWTPs range from 0.3 to 155 MGD, COD concentrations ranged from approximately 750 to 40,000 mg/L, and total silver concentrations were less than 0.6 mg/kg (1.4 mg/L) of activated sludge. After analyzing individual and total *sil* gene counts, correlations among all four genes were indicated, implying that multiple resistance genes are present in the activated sludge of each WWTP tested, likely indicating the presence of an intact *sil* generegion. Both individual and combined *sil* gene copy numbers did not correlate significantly with total silver concentration (ρ=0.176, p-value=0.548). This outcome was not surprising, as it is likely that there are other mechanisms and genes involved in conferring resistance in WWTPs that have been reported but not examined in this study. The selective pressure of antibiotics from anthropogenic sources found in both natural and engineered environments has allowed the proliferation of antibiotic resistance genes via horizontal gene transfer, and by default may have maintained the presence of the *sil* genes, as these genes are often carried on the same plasmids.

62 An Assessment of NPS Runoff Pollution in Coastal Stormwater Ponds of SC and the Potential for Development of Antibiotic Resistant Microbes

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The Southeastern US coastal plain is the most rapidly urbanizing region in the United States, with population growth exceeding 50% for most states in the region over the last 30 years. Urbanization results in landscape ecology changes which increase imperviousness and resulting alterations in the hydrological cycle, increasing runoff of nonpoint source (NPS) pollution including increased levels of nutrients, microbes and chemical contaminants such as trace metals and Polycyclic Aromatic Hydrocarbons (PAHs). Urban NPS runoff is often discharged into coastal stomwater retention ponds to reduce discharges into coastal ecosystems. In the coastal zone of SC there are > 21,594 stormwater ponds, which may concentrate chemical contaminants, nutrients and microbes often at levels much higher than in adjoining estuarine waters. An assessment of legacy pollutants and CECs was conducted in coastal region of South Carolina by examining reported sediment contaminant levels in stormwater ponds and estuarine tidal creeks. Reported sediment contaminant concentrations were compared to Sediment Quality Guidelines (SQGLs) to assess their potential to cause adverse effects on aquatic and benthic species. Also regional National Status and Trend reporting data were evaluated to assess actual toxicity data within the region with SQGL predictions. In addition, concentrations of antibiotics in WWTP effluent and surface waters were monitored along with ABR levels in bacteria at several locations in South Carolina and around the US. Results indicated that pollutant levels in SC retention ponds have shown increased levels of certain trace metals (Cd, Cr, Cu and Zn) and PAHs (Phenanthrene, Fluoranthene and Pyrene) which exceeded sediment quality guidelines and may adversely affect ecosystem conditions, including enhancement of bacterial growth and development of antibiotic resistance (ABR). Results also found the presence of several antibiotics in retention ponds that often had increased bacterial densities of *E. coli* bacteria. High rates of ABR in *E. coli* bacteria (5-22%) were found in WWTPS and CAFOs in SC and high rates of ABR resistance were found in Vibrio bacteria, as 99% were antibiotic resistant, with an average rate of antibiotic resistance (ABR) of 7.5 antimicrobials per isolate. Interactions of legacy pollutants in stormwater retention ponds will be discussed in terms how they may enhance antibiotic resistance and underlying mechanisms for upregulation of resistance genes.

63 Global Chemical Scanning of Hazards from Antibiotics, Other Pharmaceuticals and Contaminants of Emerging Concern in Aquatic Systems

B.W. Brooks, Baylor University / Environmental Science

Global concentration of human populations in cities results in unprecedented concentration of food, energy and water resource use. It also results in concentration of the use of consumer products and industrial chemicals, many of which lack robust ecological and health toxicology information. Unfortunately, in many of the urbanizing regions of developing countries, where the majority of the 22 global megacities will exist by 2030, access to and consumption of medicines and other consumer chemicals is occurring faster than environmental management systems, solid waste and sewage treatment capacity, and public health interventions occur. Our research team has advanced a unique Global Chemical Scanning approach, which employs probabilistic environmental hazard assessment (PEHA) to support synthesis of existing environmental occurrence data from chemical measures and hazard information from laboratory experiments and predictive models for pharmaceuticals and other contaminants. This presentation focuses on comparative PEHAs for select antibiotics, other pharmaceuticals and bisphenol-A to identify the likelihood of encountering exceedences of minimum selective concentrations for the development of antibiotic resistance, therapeutic hazards and predicted no effect values, respectively, from the local to global scale. Our findings identify matrices, habitats, geographic regions and chemicals requiring further environmental monitoring and surveillance, and toxicological study.

64 Antibiotics as Contaminants of Emerging Concern: Assessing Antibiotic Resistance Hazards in Aquatic Ecosystems

G.I. Scott, University of South Carolina / Env. Health Sciences; C. Horton, S. Norman, H. Scott, D.E. Porter, University of South Carolina, Arnold School of Public Health / Environmental Health Sciences; J. Moore, NOAA / NOS NCCOS / Center for Coastal Environmental Health and Biomolecular Research; P.L. Pennington, M.H. Fulton, E. Wirth, NOAA / National Centers for Coastal Ocean Science; M.I. Uyaguari Diaz, University of British Columbia / Pathobiology and Laboratory Medicine

Traditionally monitoring programs have measured legacy contaminants but not Contaminants of Emerging Concern (CECs). CECs pose many challenges in terms of monitoring and assessments (e.g. lack of analytical methods and toxicological studies). These challenges are often exacerbated because many CECs interact with metabolic pathways to produce adverse outcomes through nontraditional toxicological and molecular interactions. Antibiotics are a major class of CECs that pose significant environmental risks which may be biphasic, causing both toxic effect at high doses > Minimum Inhibitory Concentration (MIC) and development of antibiotic resistance (ABR) at doses < MIC. Antibiotics have been detected in 48% of 139 US surface water monitoring stations in national assessments. The spread of ABR has generally been attributed to the use of antibiotics in livestockand medicine, which may result in discharges from waste water treatment plants (WWTP), confined feeding animal operations (CAFOs), and aquacultural practices. The objective of this presentation will be to assess the potential hazards posed by antibiotics in the environment and factors contributing to the development of ABR. Concentrations of antibiotics were measured in WWTP effluents and surface waters along with ABR levels in bacteria (and levels of Bla_{m-1} gene) at several locations around the US. Results found the presence of several antibiotics in effluents and surface waters of the US (Triclosan and Tetracycline) that posed significant environmental risk at WWTPs and aquaculture operations, respectively. High rates of ABR in *E. coli* bacteria (5-22%) were found in WWTPs and CAFOs. The Bla_{m-1} gene was found to survive WWTP disinfection and was discharged into the marine environment, accumulating in sediments at concentrations > 1000 times higher than measured in WWTP effluent, posing potential hazards for gene transfer to other microbial species. High rates of ABR were found in Vibrio bacteria from shrimp ponds used for aquaculture and throughout the marine environment. Risk assessment methods for assessing the potential development of ABR will also be discussed including the use of the Range of MICs for determining both NOECs and ABR Potential. Also the interactions of legacy pollutants such as trace metals and physicochemical factors (salinity and temperature) in inducing ABR will be discussed.

21st Century Approaches for Capturing Diversity in Species Sensitivity to Chemicals

65 Addressing species diversity in biotransformation: Variability in expressed transcripts of Phase I and II hepatic enzymes among fishes

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There is increasing evidence that diverse xenobiotic metabolizing enzymes exist among fishes, potentially resulting in different chemical sensitivities and accumulation, but this has never been systematically evaluated. One concern is that model test species such as rainbow trout, zebrafish and fathead minnows may not adequately represent the xenobiotic metabolizing capacity of other fish species. Our current study mined available fish liver transcriptome data and performed full-transcript, isoform sequencing on liver samples from two dozen phylogenetically diverse fish species. This novel RNAseq approach eliminated the need for transcriptome reconstruction resulting in reference genomes of the highest precision, allowing for detection of enzyme isoform orthologs among the species, as well as the nuclear receptors that control expression of the enzymes. Species were selected for broad phylogenetic coverage, as well as economic, research, and conservation importance, and included: sea lamprey (*Petromyzon marinus*), lake sturgeon (*Acipenser fluvenscens*), American eel *(Anguilla rostrate)*, alligator gar *(Atractosteus spatula)*, paddlefish *(Polyodon spathula)*, rainbow trout (*Oncorhynchus mykiss*), rainbow smelt *(Osmerus mordax)*, fathead minnow *(Pimephales promelas)*, Antarctic icefish (*Trematomus loennbergii*), common carp (*Cyprinus carpio*), and channel catfish (*Ictalurus punctatus*). In addition to comparing information across fish species, the resolved isoforms were compared to human xenobiotic metabolizing enzymes. This comparison aids in evaluating the utility of human-based biotransformation tools such as ToxCast chemical screening assays or metabolism prediction software for potential relevance in fish. The content of this presentation neither constitute nor necessarily reflect USEPA policy.

66 Adaptation to cadmium reveals mechanism and susceptibility to mitochondrial genotoxicity

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Germline mutations in mitochondrial DNA have implications in ecological and human health, such as fitness, ageing, neurodegeneration, and cancer. However, mechanisms involving susceptibility to which environmental toxicants cause heritable mtDNA mutations is controversial. The microcrustacean, *Daphnia pulex* has been utilized to investigate mutation rates in previous mutation-accumulation (MA) line experiments. Until now, these have not been conducted under stress. We take advantage of a population of *D. pulex* that is tolerant to cadmium after a century of exposure to iron ore smelting runoff to investigate the mechanism of cadmium genotoxicity and mutagenesis. Twelve genetically identical individuals of sensitive (SENS) and tolerant (TOL) populations of *D. pulex* were each exposed to laboratory or chronic cadmium conditions. Genomes were sequenced after 2,000 total generations of experimental propagation to investigate mtDNA mutation rates. We find variation in base pair substitution rates: while SENS in cadmium has a lower rate than without (0.7 bp/site/generation), TOL in cadmium has almost twice the rate (1.7 bp/site/generation). Interestingly, we see very low frequencies of mutations, ranging from 3 – 10% of the population, which suggests that mutations are recent and not fixed, or that *D. pulex* retain low levels of heteroplasmy. One line of SENS in Cd shows a mutator phenotype, with a mutation rate >100-fold higher, which to our knowledge has not been observed in a mitochondrial genome. We also observed variation in rates of insertions and deletions. Understanding a mechanism behind variation in population level response may lead to better understanding of sensitivity.

67 Differences in Sensitivity to Aromatase Inhibition Among Fish Species

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There is significant concern regarding potential impairment of fish reproduction associated with exposure to endocrine disrupting chemicals (EDCs). Aromatase is a steroidogenic enzyme involved in the conversion of androgens to estrogens. Inhibition of aromatase can reduce levels of circulating estrogen leading to reduced synthesis of vitellogenin and production of fewer eggs by females. This mechanism has been extensively studied in the laboratory model species, fathead minnow (*Pimephales promelas*). However, differences in sensitivity to inhibition of aromatase among species of fish is largely unknown. This is particularly true for species that are not routinely studied in short-term reproduction assays, including many fishes of significant ecological, recreational, and economic importance such as catfish (Ictaluridae), trout (Salmonidae), and perch (Percidae). This study investigated in vitro inhibition of aromatase by the model inhibitor fadrozole across twenty phylogenetically diverse species of fish, including species with asynchronous oocyte development and species with synchronous oocyte development. Fathead minnow and other species with asynchronous oocyte development were the least sensitive in vitro with IC_{50}s of 0.1 to 0.04 µM. Synchronous species that spawn annually were more sensitive with IC_{50}s of 0.02 to 0.007 µM. Sturgeons (Acipenseridae) and paddlefish (Polyodontidae) that have synchronous oocyte development but spawn less than annually were the most sensitive with IC_{50}s of 0.002 µM. This suggests that intrinsic differences in sensitivity to inhibition of aromatase could be as great as 50-fold among fishes. However, species with the same process of oocyte development had comparable in vitro sensitivities to inhibition. Therefore, knowledge of reproductive strategy might be predictive of relative sensitivity, with species with asynchronous oocyte development being less sensitive than species with synchronous oocyte development. Results of this study are being used in the construction of a quantitative adverse outcome pathway that incorporates species-specific differences in sensitivity to inhibition of aromatase for application to the regulation and risk assessment of EDCs that can inhibit aromatase. The content of this presentation neither constitute nor necessarily reflect USEPA policy.

68 Comparative behavioral toxicology of two common larval fish models

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Behavioral studies can offer insight into chemical modes of action and present an opportunity to begin to investigate linkages among chemical bioactivity, a molecular initiation event, and potential behavioral response patterns associated with adverse outcomes. These responses inform toxicology studies by rapidly and sensitively detecting molecular initiation events that propagate to physiological changes in individuals. Behavioral thresholds for a wide range of contaminants often manifest at lower chemical concentrations than those eliciting mortality and other standardized adverse outcomes. The larval zebrafish is a common model in behavior, developmental toxicology, neurotoxicology and other biomedical studies. Whereas the fathead minnow is a common model for aquatic toxicology research and regulatory programs, and as a model has received comparatively little attention in behavioral studies. We employed the zebrafish and fathead minnow models to define toxicant induced swimming activity alterations during interchanging photoperiods. We specifically compared behavioral responses among eight electrophilic reactive compounds (3-bromo-1-propanol, 3-chloro-1,2-propanediol, dibromoacetonitrile, glycidol, sodium decyl sulfate, styrene oxide, tris(2,3-dibromopropyl) phosphate (TBPP), and tris(1,3-dichloro-2-propyl) phosphate (TDCPP)) in the USEPA ToxCast Program. Following OECD FET and EPA WET experimental guidelines, zebrafish embryos and fathead minnow larvae were exposed for 96 h to each compound, then observed using a digital behavioral analysis system (ViewPoint). Behavioral observations occurred for 50 minutes (a 10-minute acclimation period, two 10-minute dark periods, and two 10-minute light periods). The fish models demonstrated differential response patterns to changing photoperiods. Fathead minnow activity dramatically increased with a sudden change to light, while zebrafish activity decreased during light photoperiods and increased during dark photoperiods. The eight chemicals selected from the USEPA ToxCast program elicited a diverse array of behavioral responses in fish larvae.

69 Physiological basis of life-stage and species-specific differences in the acute sensitivity of rainbow trout and white sturgeon to waterborne cadmium

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Fish exhibit wide variations in life-stage and species-specific sensitivity to metals. The high diversity found in fish species makes it challenging to test every metal and fish combination to estimate the differences in sensitivity. At present, the physiological mechanisms underlying the species and life-stage specific differences in metal sensitivity in fish are poorly understood. Such knowledge is important since it can be useful in developing predictive models for metal sensitivity assessments in fish. In the present study, we demonstrated that rainbow trout and white sturgeon are two disparate fish species with substantial life-stage (larval, swim up and juvenile) and species-specific differences in acute waterborne Cd sensitivity, with trout being generally more sensitive than sturgeon. The acute toxicity (96h LC_{50}) of Cd was similar in both species at the larval stage (12.52 and 17.5 µg/L, respectively). However, rainbow trout demonstrated an increased sensitivity to Cd in later life-stages (96h LC_{50}: 1.93–3 µg/L), whereas an opposite trend was observed in white sturgeon (96h LC_{50}: >80 µg/L in juvenile stage). We also found that despite of their lower sensitivity to Cd, white sturgeon tend to accumulate greater amount of Cd than rainbow trout at each life-stages (up to 9 times). Moreover, the evaluation of the Cd uptake kinetics across life-stages and species demonstrated that generally white sturgeon had a lower affinity (inverse of Km) and a higher maximum uptake rate (Jmax) for Cd relative to rainbow trout across life-stages (Km - 140– 170 nM and 60–70 nM, Jmax - 0.3–0.4 and 0.2–0.3 nM/g wet wt/h in white sturgeon and rainbow trout, respectively). The gradual increase in short-term (3h) waterborne Ca (a Cd anatgonist) and Cd uptake from the larval to later life-stages corresponded with an increased expression of Epithelial Calcium channel (ECaC) gene in the gills of both species. We also observed that the apparent difference in both species-specific and life-stage specific Cd sensitivity could be explained, at least in part, by the relative difference in the magnitude of Cd-induced inhibition of branchial Ca uptake and loss of whole body Ca level. Overall, our findings suggest that species and life-stage specific differences in sensitivity to Cd (and possibly other metals) depend on the interplay among several toxicokinetic and toxicodynamic parameters.

70 Sensitivity of aquatic invertebrates towards the pyrethroid insecticide cypermethrin

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The pyrethroid insecticides are widely used in agriculture for control of a range of different insect pests and constitute a large percentage of the global insecticide usage. Pyrethroid insecticides have, however, been found in surface waters. In particular, the transport of pyrethroids is governed by surface runoff and flow through tile drains during heavy precipitation incidents leading to transient pulse exposures of stream organisms. Importantly, recent studies have shown that even short pulses of pyrethroids are highly toxic to different aquatic invertebrates with EC_{50}-values in the ng L^{-1} range. The purpose of the current study was to test if the mortality based concentration-response relationship induced by a 24 hour pulse of the pyrethroid insecticide cypermethrin exposed to 11 species of aquatic invertebrates could be explained and modeled by different toxicokinetic and toxicodynamic (TKTD) parameters such as uptake and elimination rates as well as scaled damage or internal concentrations as described under the GUTS-framework. We finally measured the in vivo cytochrome P450 (ECOD) activity of the test organisms as a rough surrogate for their in vivo biotransformation capabilities of xenobiotics to see if this could explain the pattern of toxicity. More than a 7,000 fold difference was observed for the EC_{50}-estimates between the most sensitive (*Chaetopteryx villosa*; 0.08 ± 0.09 µg L^{-1}) and the least sensitive (*Tubificidae*; 546 ± 1562 µg L^{-1}) species. The TKTD models assuming stochastic death (SD) described the survival data slightly better than the models assuming individual tolerance (IT), however, for future studies a higher time resolution is recommended to provide more data points for the models. Interestingly, did the in vivo cytochrome P450 (ECOD) activity vary quite significantly also between the species with similar sensitivities towards cypermethrin. Despite that the uptake and eliminations rates still need to be determined the current study showed that TKTD models under the GUTS-framework can model and describe toxicity of an insecticide towards a broad range of aquatic invertebrates.

71 Developing population models for pesticide risk assessment: A systematic approach using the example of herbaceous plants

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The sensitivity of populations to stresses from chemical exposure is not linearly related to sensitivities of individuals, but depend on a species' life history, population dynamics, and various other factors. Population models provide a means to assess stressors in the context of population-level dynamics, species and habitat characteristics. They are increasingly recognized as important tools in pesticide risk assessment and were recently identified as essential for endangered species assessment in the U.S. However, few population models for this specific purpose have been developed to date. Developing such models in a systematic and transparent way would increase their applicability and credibility and reduce development efforts. We present a systematic and transparent approach to developing population models. The guidance informs the model developer on necessary steps that consider the specific questions to be addressed by the model through four phases. In the first phase, the model developer systematically reviews details of the model objectives. Data available for the modeled species and stressor(s) are compiled in table format during the second phase. Starting with a conceptual model of the species' life history in the third phase, seven decision steps guide the model developer through decisions on what and how details should be represented in the model based on the model objectives and data availability. Decision steps may need to be revisited iteratively during the third phase. In the fourth phase, the model developer compiles a summary of the conceptual model including any underlying assumptions. Uncertainties arising from data and model assumptions are also explicitly characterized. We provide an example decision guide for the development of population models of herbaceous plants applied in pesticide risk assessment. We emphasize how different species' characteristics are represented in population models, and how they can inform species-specific chemical risk assessment. The adaptation of the approach to developing population models for other taxonomic groups and applications will be discussed.

72 Evaluating the Role of Fish as Surrogates for Amphibians in Ecological Risk Assessment

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Ecological risk of chemical exposure to aquatic-phase amphibians is historically evaluated using surrogate toxicity data from standard fish species. Recently published meta-analyses of fish and amphibian ecotoxicity data concluded that both groups are similarly sensitive to a range of chemicals. However, these analyses are limited because the amphibian data reported in the peer-reviewed literature are variable both with respect to experimental design and test species. In 2010, the U.S. Environmental Protection Agency began receiving ecotoxicity data for a standard amphibian test species (Xenopus laevis) as part of the Endocrine Disruptor Screening Program. Although these studies are primarily designed to inform a determination of potential thyroid interaction within the context of other endocrine screening studies, they also contain valuable data on survival and growth that can be compared to existing fish data for a given chemical. We used this dataset to compare no observed adverse effect concentration (NOAEC) values for survival, body weight, and length data between fish and amphibians for 43 different pesticide active ingredients. Overall, the results indicate that fish are a reasonably good predictor of amphibian toxicity as there were no statistically significant differences in NOAEC values between the two groups for the endpoints examined. However, toxicity endpoints were lower in amphibians as compared to fish approximately half the time across chemicals, challenging the notion that fish are consistently more sensitive than amphibians. The views expressed in this abstract are those of the authors and do not represent Agency policy or endorsement.

Implementation of TSCA as Amended by the Frank R. Lautenberg Chemical Safety for the 21st Century Act – Science Issues

73 Implementation of TSCA as Amended by the Frank R. Lautenberg Chemical Safety for the 21st Century Act - Science Issues

T.R. Henry, USEPA / Risk Assessment Division Office of Pollution Prevention and Toxics

On June 22, 2016, the Frank R. Lautenberg Chemical Safety for the 21st Century Act which amends the Toxic Substances Control Act (TSCA), the Nation's primary chemicals management law was signed into law. The amended TSCA includes several new mandates, including: (1) a mandatory requirement for EPA to evaluate existing chemicals with clear and enforceable deadlines; (2) assessments are risk based without consideration of economic or non-risk factors; (3) increased public transparency for chemical information; and (4) a consistent source of funding for EPA to carry out the responsibilities under the new law. Several of the new provisions of TSCA require additional science-based approaches be developed, advanced and/or applied. Under section 4 of TSCA, EPA must reduce and replace, to the extent practicable and must encourage and facilitate the use of scientifically valid test methods and strategies that will support regulatory decisions. Under section 5 of TSCA EPA must make an affirmative finding with regard to unreasonable risk, often with very limited information available, which requires application of predictive models and estimation approaches and/or requests for testing when information is insufficient to make a reasoned evaluation. Section 6 of TSCA requires that EPA develop a risk-based screening process to identify high-priority and low-priority chemicals for further risk evaluation. This prioritization process must include considerations of hazard, exposure potential, persistence and bioaccumulation among other things. Risk evaluation must be conducted for all chemicals designated high-priority and must integrate and assess available information on hazards and exposures for the conditions of use of a chemical substance. Furthermore, EPA must also apply a number of scientific standards in carrying out sections 4, 5 and 6 of TSCA, including to use scientific information in a manner consistent with the best available science and consider as applicable reasonableness, relevance, clarity and completeness, variability and uncertainty, and peer review. EPA's progress in implementing these provisions will be presented.

74 Empirical and Modeling Methods for Exposure Assessment under Amended TSCA

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The U.S. Environmental Protection Agency's Risk Assessment Division, in the Office of Pollution Prevention and Toxics, conducts chemical risk evaluations in accordance with the Toxic Substances Control Act (TSCA). Amendments to TSCA were signed into law on June 22, 2016 as the Frank R. Lautenberg Chemical Safety for the 21st Century Act. Risk evaluations mandated under amended TSCA are required to determine whether a chemical presents an unreasonable risk to humans or the environment. The implementation of amended TSCA affects both new and existing chemical assessments, and has important implications for exposure assessment. Evaluations of environmental exposure include consideration of the conditions of use over the lifecycle of a chemical in commerce, including environmental releases, fate in the environment, and hazard identification. There are a variety of methods, models and data sources that may be used to quantify environmental exposure. Measured properties and monitoring data, alone or in conjunction with mathematical modeling approaches, inform exposure scenarios. Information such as physical-chemical and fate properties, chemical release to the environment and environmental media concentrations provide necessary context to build environmental exposure assessments. Amended TSCA mandates that EPA evaluate existing chemicals under clear and enforceable deadlines, using risk-based safety standards. In June of 2017, EPA released the intended scope of evaluation for the first round of ten existing chemical evaluations. This talk will describe the integrative data collection and modeling approaches for assessing environmental exposures undertaken in the "first 10" post-amendment existing chemical scope documents. The views expressed in this abstract are those of the authors and do not represent Agency policy or endorsement.

75 Environmental Hazard Assessment of Low Molecular Weight Oligomeric Components of Polymers

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The Frank R. Lautenberg Chemical Safety for the 21st Century Act was signed into law on June 22, 2016, thereby amending the Toxic Substances Control Act (TSCA), the Nation's primary chemicals management law. Implementation of the amended legislation is carried out by USEPA's Office of Pollution Prevention and Toxics and includes environmental hazard assessment of industrial chemicals, including polymers with an average molecular weight (MW) greater than 1000 daltons. Often, little or no environmental toxicity data are available to characterize environmental hazards of these chemicals and predictive QSAR hazard assessment methods such as ECOSAR are not recommended if the MW of a polymer exceeds 1000 daltons. As a result, polymers may be assessed differently than other discrete chemicals. To affirmatively conclude whether a polymer is likely to present an unreasonable risk of injury to health or the environment, all parts of polymer need to be assessed. This includes assessing the polymer, as well as the low molecular weight (LMW) oligomeric components of the polymer (MW< 1000 daltons), including all reactive monomers and functional groups. LMW oligomeric components may be assessed using conventional hazard assessment methods (e.g., QSARS, analogs, read-across). This presentation will discuss current approaches for the environmental hazard assessment of LMW oligomers, their strengths and limitations, and current data needs. This presentation will also outline the role of LMW oligomeric components in the hazard assessment process for polymers. Disclaimer: The views expressed in this abstract are those of the authors and do not represent Agency policy or endorsement.

76 Testing Requirements for Photo Acid Generators in the New Chemicals Program under Amended TSCA

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The evaluation of new chemical substances under the amended Toxic Substances Control Act (TSCA) represents a screening-level process to identify those chemicals that may present an unreasonable risk of injury to health or the environment. Due to the paucity of data available for the wide variety of chemical substances manufactured or imported in the U.S., these screening-level assessments often include assumptions that are informed from data on groups of chemical substances. When new data become available, EPA's default assumptions may need to be re-evaluated. Recent data have led EPA/RAD to re-evaluate the assumption that Photo Acid Generators (PAGs) released to the environment will exist as tight ion pairs and do not dissociate under environmental or biological conditions. Potential risk from tightly bound ion pairs with low water solubility and low bioaccumulation potential is expected to be lower than that of disassociated ionic chemical species. Data, including 44 submitted studies on 19 different PAG chemicals and open literature information, indicates higher than expected environmental toxicity. These data suggest moderate to high hazard to aquatic invertebrates and algae. Based on these data, EPA hypothesized that PAGs may be more toxic and bioaccumulative than previously assumed either in the parent form or from the component ions and/or possible degradants. EPA is therefore no longer assuming low ecological hazard, persistence, and bioaccumulation potential for PAGs for new chemical substance submissions. Instead EPA is implementing a tiered PAGs testing strategy to require generation of key data needed for risk assessment. This tiered testing strategy is designed to understand specific endpoints (i.e., exposure and release pathways [Tier 1], physicochemical properties and fate [Tier 2], environmental hazard and bioaccumulation [Tier 3a], and human health hazard [Tier 3b]) for potential exposures or releases that may occur during the lifecycle of PAGs, the component ions, and/or degradants. This presentation describes the rational for EPA's proposed testing strategy for PAGs submitted to the Agency's New Chemicals Program under the amended TSCA. Disclaimer: The views expressed in this abstract are those of the authors and do not represent Agency policy or endorsement.

77 Prioritization of Literature Search and Review Using Natural Language Processing for Existing Chemicals Risk Evaluations under the Amended TSCA

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The amended Toxic Substances Control Act (TSCA) requires EPA to develop fit-for-purpose risk evaluations to determine whether a chemical substance presents an unreasonable risk of injury to health or the environment. To meet these requirements, EPA is implementing systematic review across various multi-disciplinary lines of evidence supporting the risk evaluation (i.e., exposure, fate, ecological hazard, and human health hazard). These evaluations also necessitate comprehensive literature searches to identify relevant data, but these searches often require manual review of thousands of results. Natural language processing (NLP) and text analytics can increase efficiency in reviewing results and by prioritizing data for review that are most likely to be relevant. In this presentation, we describe the initial data gathering method for the first 10 existing chemicals evaluated under amended TSCA. We compare this approach to the results expected for the same chemicals using natural language processing techniques. Specifically, we focus on utilizing clustering techniques that sort references into topical groups, or clusters. We first demonstrate the efficacy of NLP by implementing supervised clustering with a "seed set" of known relevant, chemical-specific references to prioritize comprehensive search results; we then compared the clustered results to manual screening results in terms of total number of studies to screen manually and number of relevant studies retrieved. However since this approach requires a set of known relevant references for each chemical that might not be easily obtained for future chemicals, we also explored if it is possible to create a chemical-agnostic seed set that could be applied to future chemical assessments by comparing supervised clustering predictions using a generic seed set of studies. Lastly, we demonstrate the utility of applying machine learning following supervised clustering to maximize search precision and recall in the literature database to minimize the level of effort in review while maximizing the number of relevant references reviewed. These test cases demonstrate that natural language processing provides an opportunity to reduce the level of effort required to review references while ensuring literature searches are comprehensive. The views expressed in this abstract are those of the authors and do not represent Agency policy or endorsement.

78 The role of non-animal safety assessment methods in implementation of the new TSCA

C. Willett, the Humane Society of the United States / Animal Research Issues

The Frank R. Lautenberg Chemical Safety for the 21st Century Act was signed into law by President Obama and came into force on June 22, 2016. This amendment to TSCA increases the authority of the Environmental Protection Agency to obtain information on both new and existing industrial chemicals and, in addition to requiring the use of the "best available science," the amendment includes a section requiring both EPA and "any person" developing information under the Act to reduce and replace vertebrate testing "to the extent practicable, scientifically justified, and consistent with the policies of this title." This section of the bill also requires EPA to "promote the development and timely incorporation" of non-vertebrate animal methods by developing a strategic plan to do so and to publish and update regularly a list of acceptable methods and approaches. Under amended TSCA, existing chemicals will be subject to prioritization via a risk-based screening process into high and low priority. High priority chemicals ("those that may present an unreasonable risk" or for which there is insufficient information to make a determination) must undergo a risk evaluation. New chemicals will all be subject to review, and if EPA cannot make a determination regarding safe use, may ask for additional information. The amended Act is therefore likely to require the generation of massive amounts of new information on both existing and new industrial chemicals; at the same time there is pressure to reduce vertebrate testing, creating an immediate need for increased implementation of non-vertebrate evaluation tools. Such rapid development and implementation will require strongly coordinated efforts between industry, agency scientists and regulators, and other stakeholders to leverage exiting approaches form other sectors and expand available methods and approaches. This presentation will offer some suggestions for immediate action.

79 In Vitro Metabolomics as Alternative Testing Strategy for Predicting Adverse Outcome Pathways

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Toxicology in the 21st century continues to move towards a new paradigm in testing and risk assessment. Under the amended Toxic Substances Control Act (TSCA), EPA is exploring methods and approaches to better understand and estimate hazard, exposure and risk. Advances are being developed and refined to better predict chemical risk from in vivo and in vitro studies. These advances include: (1) deep sequencing of genomic regulatory regions or micro array data coupled with high throughput mass spectrometry; and (2) data from metabolic studies providing opportunities to construct models for prediction of molecular initiating events (MIEs) and key events (KEs). These technologies may be used to build our understanding of Adverse Outcome Pathways (AOPs). Other technologies we are exploring include: (1) genetic manipulations (gene KOs, transgenics-pathway perturbations) deciphering key events in known metabolic pathways; and (2) gene expression biomarkers that accurately predict activation of xenobiotic transcription factors (TFs) such as constitutive activated receptor (CAR), aryl hydrocarbon receptor (AhR), peroxisome proliferator-activated receptors (PPARs), and corticosteroid receptors (ER, AR). These biomarkers could be used to comprehensively assess MIE/KE modulation in risk assessment for chemicals prioritized to be assessed under TSCA. Using these new tools/data for risk analysis may highlight a number of features due to the chemical exposure, *e.g.*, if similar classes of chemicals can activate multiple MIEs. However, simultaneous assessment of known AOPs may uncover unexpected key event relationships (KERs) between TFs (e.g., synergistic induction of CYP genes by CAR and HNF4a, potentiation of chromatin accessibility by AP-1 and GR). *In vitro* models such as Rainbow trout liver hepatocytes (RT-HEP) and S9 (RT-S9) fraction would also be helpful as alternative testing methods, which is a key requirement for risk evaluation in the amended TSCA law. Incorporating epigenomics and metabolomics markers would help in better dissecting the still missing exposoure mechanistics for most chemicals classes. Integration of several types of data, from environmental exposures to epigenetics, metabolomics and genomics, requires the use of innovative bioinformatics and data reduction techniques and such integrated predictions may better inform assessment of risk using AOPs. The views in this abstract are those of the authors and do not represent USEPA policy.

80 Incorporating High-Throughput in Vitro Bioactivity and Toxicokinetics with Predicted Exposure to Advance Screening-Level Human Health Risk Assessment

K. Paul Friedman, USEPA / National Center for Computational Toxicology; R.S. Thomas, USEPA / Office of Research and Development / National Center for Computational Toxicology

Tens of thousands of chemicals are currently in commerce, and hundreds more are introduced every year, but only a small fraction of chemicals have been adequately evaluated for potential human health effects due to resource-intensive methods. Use of high-throughput, in vitro bioactivity data to estimate point-of-departures (PODs) has the potential to accelerate human health risk assessments. In the USEPA's ToxCast program, high-throughput in vitro assays broadly characterized the biological activity and potential mechanisms of ~1,800 chemicals. *In vitro* toxicokinetic assays and in vitro-to-in vivo extrapolation modeling enable conversion of in vitro bioactive concentrations to estimated administered dose equivalents (mg/kg/day). The USEPA's ExpoCast project uses key aspects of chemical production, fate, transport, and personal use to predict human exposure. Advancement toward the goal of accelerated risk assessment by incorporated high-throughput predictions of bioactivity, toxicokinetics, and exposure for risk assessment will be discussed using a case study. PODs predicted using high-throughput methods and PODs from traditional animal toxicology studies were compared to high-throughput predictions of exposure for ~400 chemicals. The results demonstrate the feasibility, and continuing challenges, of using high-throughput predictions of bioactivity to estimate PODs in screening level assessments. This abstract does not necessarily reflect USEPA policy.

Recent Advances and Future Direction of Per- and Polyfluoroalkyl Substances (PFASs) Research

81 Pilot-Scale Application of Granular Activated Carbon for Removal of Per- and Polyfluoroalkyl Substances in Groundwater at a Military Site

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Aqueous film-forming foams (AFFFs) have historically been applied to hydrocarbon-based fuel fires because of their unique, proprietary chemistries and, as a result, are used for both emergency response and fire-fighter training purposes. Repeated use of AFFFs at fire-fighter training areas resulted in contamination of soils, aquifer sediments, and groundwater with PFASs including perfluoroalkyl carboxylates (PFCAs, e.g., PFOA) and sulfonates (PFSAs, e.g., PFOS) at concentrations above current health advisory levels. Granular activated carbon (GAC) is one remediation technology used to remove PFASs from groundwater. At present, there are few data at pilot and full scale that describe the removal of PFASs by GAC. With the focus primarily on the removal of PFOA and PFOS, there are few data that describe the removal of other PFASs by GAC, including precursors. A pilot-scale system consisting of flow-through lead and lag vessels, each containing 91kg DSR-GAC, and was operated at a flow rate of approximately 9.5 liters per minute. Weekly samples of influent and the effluents from the lead and lag vessel were analyzed for 16 individual PFASs including C4-C8 PFCAs, C4-C8 PFSAs, 4:2-8:2 fluorotelomer sulfonates, C6 and C8 perfluoroalkyl sulfonamides, and a cationic precursor. Samples were also analyzed by the total oxidizable precursor (TOP) assay and by particle induced gamma ray emission (PIGE) spectrometry for total fluorine. Individual PFASs that are oxidizable accounted for the production of PFCAs upon oxidation, thus closing the mass balance on PFASs in the influent groundwater. Breakthrough curves were constructed to determine the efficacy for removal of the individual PFASs. In addition, the relationship between the order of individual PFAS elution on analytical columns used in LC-MS/MS analysis was determined to be the same as the order of breakthrough on GAC. The relationship between elution and breakthrough could be used for predicting breakthrough of other precursors. Breakthrough curves were also generated from TOP assay and PIGE data. Discussion will include the advantages and limitations of relying on TOP assay and PIGE data for total fluorine as an alternative or in addition to individual PFAS analysis by LC-MS/MS.

82 Sorption of PFASs relevant to AFFF-impacted Groundwater by Biochars and Activated Carbon

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Despite growing concerns about human exposure to perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS), other poly- and perfluoroalkyl substances (PFASs) derived from aqueous film-forming foams (AFFFs) have garnered little attention. While these other PFASs may also be present in AFFF-impacted drinking water, their removal by conventional drinking water treatment is poorly understood. This study compared the removal of 30 PFASs, including 13 recently discovered PFASs, from an AFFF-impacted drinking water using carbonaceous sorbents (i.e., granular activated carbon, GAC). The approach combined laboratory batch experiments and modeling: batch sorption data were used to determine partition coefficients (Kd) and calibrate a transport model based on intraparticle diffusion-limited sorption kinetics, which was used to make forward predictions of PFAS breakthrough during GAC adsorption. While strong retention was predicted for PFOS and PFOA, nearly all of the recently discovered polyfluorinated chemicals and PFOS-like PFASs detected in the AFFF-impacted drinking water were predicted to breakthrough GAC systems before both PFOS and PFOA. These model breakthrough results were used to evaluate a simplified approach to predict PFAS removal by GAC using compound-specific retention times on a C18 column (RTC18). Overall, this study reveals that GAC systems for treatment of AFFF-impacted sources of water for PFOA and/or PFOS likely achieve poor removal, when operated only for PFOS and PFOA removal, of many unmonitored PFASs of unknown toxicity.

83 Association of perfluoroalkyl acids (PFAAs) and maternal thyroid hormone status: A longitudinal assessment of gestation and postpartum relationships

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Perfluoroalkyl acids (PFAAs) are persistent organic contaminants that are associated with the disruption of thyroid hormone (TH) homeostasis. Maternal TH status is critical for healthy fetal brain development during pregnancy, particularly during the early stages when the fetus is entirely dependent on a maternal supply of THs. The aim of the investigation was to determine if maternal PFAA exposure is associated with altered TH levels measured at multiple time points in a Canadian birth cohort while considering thyroid-specific conditions and the potential for mixture effects from mercury co-exposure. Maternal blood samples (n = 501) were collected at 3 trimester-specific timepoints and at three months postpartum from participants enrolled in the Alberta Pregnancy Outcomes and Nutrition (APrON) birth cohort. Second trimester plasma or blood samples were analysed for PFAAs or mercury and mixed effects regression models were used to investigate potential associations with THs. Thyroid peroxidase antibodies, a marker of autoimmune hypothyroidism, and mercury were both included in PFAA-TH models as interaction terms. Perfluorohexane sulfonate (PFHxS) and isomers of perfluoroctane sulfonate (PFOS) were both significantly positively associated with thyroid stimulating hormone (TSH). Moreover, these associations were time dependent, with the greatest effect early in pregnancy and diminishing as pregnancy progressed. In addition to these findings, PFHxS was observed to be negatively associated with free thyroxine (FT4) and significant differences were found for numerous PFAA-TH associations when comparing outcomes during gestation to post-pregnancy. The addition of TPOAb and mercury into statistical models did not significantly alter these associations. These results suggest that specific PFAAs in the plasma of a background Canadian population are high enough to significantly influence maternal thyroid hormone homeostasis in the most highly exposed individuals. The strongest association was found for PFHxS, the strongest binding PFAA to transthyretin in vitro. The associations of PFAAs and thyroid hormones were found to be trimester-dependent, and may explain discrepancies between findings in published literature.

84 PFOA and its Substitute GenX Found in the Environment around a Teflon Production Site in The Netherlands

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Between 1970 and 2012 Du Pont, now named Chemours in Dordrecht, The Netherlands, has used perfluorinated octanoic acid (PFOA) as an intermediate in their Teflon production. PFOA is highly persistent, bioaccumulative, toxic, ubiquitously present in the environment and listed by the European Chemical Agency as a substance of very high concern. In 2014,the European Union proposed to ban its use and production. Manufacturers have, therefore, changed their production and moved towards alternative fluorinated compounds for Teflon production. At the plant in Dordrecht, GenX was introduced to replace PFOA . GenX or FRD-903 is the commercial name for the ammonium salt of 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (HFPO-DA). GenX was selected because of a considerable shorter half-life in humans. Its toxicity is comparable to that of PFOA. However, Gen X has a much better water solubility compared to PFOA. Consequently, GenX has been detected in surface water (70-150 ng/L) and meanwhile also in drinking water (20 ng/L) around Dordrecht, provided to 750,000 inhabitants. This concentration in drinking water exceeds that of PFOA in the same drinking water. We carried out a first screening in grass and mayflower leaves around (50-3000m) the Teflon production site and found substantial levels of both PFOA and GenX. Detection of PFOA and GenX was performed by LC-MS/MS (Bruker EVOQ TQ), using ^{13}C labeled standards. GenX was detected in the grass and the leaves at 700 m from the factory, with levels ranging from 0.2 -1.5 µg/kg in the grass and from 1.1-4.7 µg/kg in the leaves. Further away from the factory, at 3 km, GenX levels were below the limit of detection (< 0.06 µg/kg). A decreasing concentration in both the grasses and the leaves was observed for GenX with distance from the factory. Highest levels were detected in the leaves and the samples collected near the factory. The results indicate that the factory is a point source for the emission of GenX. Similar patterns were observed for PFOA. However, the PFOA levels were approximately 5-8 times higher compared to the GenX levels, most likely due to the longer period PFOA has been in use. Possibly, PFOA may be transferred from the ground and groundwater to the grass or the mayflower bushes. Meanwhile the local authorities have limited the permit for the release of GenX by Chemours from 6400 kg/yr to 2035 kg/yr.

85 Identification of New Non-ionic, Cationic, Zwitterionic, and Anionic Polyfluorinated Substances

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Poly- and perfluoroalkyl substances (poly- and per-PFASs) are a large group of organic compounds that have become the target of investigation due to their widespread occurrence in the environment and biota, coupled with their known or suspected impacts on human health. Recent studies have shown that a significant portion of poly-PFASs remain unidentified. This study presents a time-of-flight mass spectrometry approach based on continuously interleaving scans at low and high collision energies (ToF-MS^E) for the rapid identification and characterization of unknown PFASs. The MS^E mode allowed for the simultaneous acquisition of full-spectrum accurate mass data of both parent and fragment ions in a single chromatographic run. Specific to PFASs, the hypothesis that PFASs can be selectively detected by the ToF–MS^E high-resolution parent-ion search HRPIS was confirmed with 24 certified poly- and per-PFAS standards. After being validated with the PFAS standards, the innovative HRPIS approach was applied to a group of commercial surfactants, which led to the identification of 42 new and 38 infrequently reported PFASs including 32 non-ionic, 30 cationic, 15 zwitterionic, and 5 anionic compounds.

It is envisaged that the results, especially the identification of non-ionic PFASs, may provide important insights into the historical occupational and non-occupational exposure to PFASs from the production and application of these surfactants.

86 Contemporary and temporal investigation of per- and polyfluorinated compounds in Cape Fear River, North Carolina surface water samples

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Recent regulatory pressure has altered the chemistry of per- and polyfluorinated compounds being manufactured and used in industrial and consumer applications. Many manufacturers have been moving toward the production of shorter chain per- and polyfluorinated compounds. A series of polyfluorinated compounds that contain central ether oxygens have been recently documented in the peer reviewed literature to be present in the Cape Fear river, NC in both surface and drinking water samples. Non-targeted analysis of water samples using high resolution mass spectrometry (HRMS) LC/MSD TOF was used in the past to identify novel polyfluorinated compounds. Contemporary samples were collected recently to: 1) confirm the presence of previously identified chemicals 2) investigate novel chemicals present 3) analyze via TOFMS and QTOFMS for platform cross validation 4) retroactively investigate samples from over 5 years past. Contemporary TOFMS/QTOFMS analysis revealed the presence of a series of polyfluorinated ether sulfonic acids that were previously undescribed. Precursor compounds were selected from a list of molecular features (accurate mass, retention time, abundance) that were unknown. QTOFMS data dependent analysis (DDA) was performed on select precursors to generate fragmentation spectra. One advantage of HRMS and proper data banking is retrospective investigation of past samples. This presentation will focus on TOF/QTOF based analytical approaches used to identify novel chemicals species and temporal occurrence of detected compounds.

87 Development, Alternatives Assessment and Value-in-Use of Short-Chain Fluorotelomer-based Products for Textiles, AFFF, Carpets and Other End-Uses

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Per- and polyfluoralkyl substances (PFASs) is a term that describes a wide and diverse array of chemistry containing fluorine and carbon. The focus of this presentation will be on one group of PFASs, fluorotelomer-based products. Fluorotelomer-based products can be either polymeric or non-polymeric. One significant use for fluorotelomer-based products is providing stain, oil and water repellency on fabrics. These fluorinated repellent products, including durable water repellents (DWRs), are usually polymeric fluorotelomer-based products that are typically applied in combination with other finishing auxiliaries. Non-polymeric fluorotelomer based products are used in multi-component formulations called Aqueous Film Forming Foams, AFFF, which provide unmatched Class B (i.e., hydrocarbon & polar solvent liquids) fire fighting performance. While these polymeric fluorotelomer-based products perform well and provide essential performance on garments and the non-polymeric fluorotelomer-based products provide superior fire-fighting in AFF performance, each of these chemistries have the potential to be released and could create an environmental footprint. The remarkable strength of the C-F bond provides superior resistance to extreme thermal, chemical and environmental conditions. While this uniquely high stability makes these products ideal to protect people, equipment and property, it also makes them resistant to degradation and persistent in the environment. This poster/presentation will highlight the development of the newest generation of short-chain fluorotelomer-based products. A comprehensive assessment of their toxicological properties, regulatory status, and an overview of their value-in-use including some essential uses, will be discussed in detail. An industry perspective on when and how best to use these products while at the same time minimizing the environmental footprint will also be featured in this presentation/paper.

88 A systematic study of the binding of varying chain length perfluoroalkyl acids to human serum albumin

L.A. MacManus-Spencer, J. Ulrich, Union College / Chemistry

Perfluoroalkyl acids (PFAAs), a subset of poly- and perfluoroalkyl substances (PFAS), are used in the manufacture of a wide variety of consumer products, as well as in fire-fighting foams. PFAAs are persistent and pervasive in the environment, bioaccumulate in organisms, and are toxic. Unlike most other hydrophobic organic contaminants, which accumulate in fatty tissue, PFAAs preferentially accumulate in the blood, liver and kidneys, tissues with high protein content. Therefore, bioaccumulation models that utilize octanol-water partition coefficients (K_{OW}) are unsuitable for predicting PFAA bioaccumulation. PFAA-protein binding constants are needed in order to better understand the pharmacokinetics and tissue distribution patterns of PFAAs. In previous studies, researchers have published data on the binding of the so-called “long-chain” PFAAs (i.e., perfluorooctanoate, PFOA, and perfluorooctane sulfonate, PFOS) to serum albumin, the most abundant protein in the blood of many organisms that also transports endogenous and exogenous ligands throughout other tissues of the body. However, from the perspective of human health, the limitations of these studies include the focus on only PFOA and/or PFOS (as opposed to the variety of PFAS found in the environment, including the newer short-chain PFAA replacement chemicals), the use of bovine or rat serum albumin in some studies, and a lack of consistency in the bioanalytical methods used. In this study, an updated, systematic equilibrium dialysis method was used to quantify the binding of PFAAs with varying chain length (four to twelve carbons) and ionic head group (carboxylates and sulfonates) to human serum albumin (HSA). Our results indicate that a two-class binding model best describes the HSA binding of all PFAAs studied. Binding constants for the high-affinity sites are on the order of 10^4-10^6 M^{-1}, and those for the low-affinity sites are on the order of 10^2-10^4 M^{-1}. In general there is an increase in binding affinity at both types of sites with increasing chain length, with the sulfonates displaying a more pronounced increase per CF_2 unit. However, perfluorobutanoate (PFBA, a replacement for PFOA) binds as strongly to HSA as PFOA at the high-affinity sites, and perfluorobutane sulfonate (PFBS, a replacement for PFOS) binds only moderately weaker to HSA compared to PFOS at the high-affinity sites. This raises concern about the continued use of the short-chain replacement PFAAs in consumer products.

Expanding Beyond the Honey Bee – Novel Approaches for Advancing Risk Assessment for Non-Apis Bees

89 Extrapolating acute toxicity across bee species - the influence of size

H. Thompson, Syngenta Ltd / Environmental Safety; J. Overmyer, Syngenta Crop Protection, LLC. / Environmental Safety

Apis mellifera has been widely used globally as a model species in pollinator regulatory risk assessment. OECD guidelines for test methods to generate data for regulatory use have been in place for 20 years with the aim of ensuring comparability. More recently, there has been increasing interest in the toxicity of pesticides to a wider range of pollinators and in factors which can be used for extrapolating pesticide toxicity from *Apis mellifera* to other bee species to ensure risk assessments are protective. Although a 10-fold safety factor for toxicity has been proposed by the European Food Safety Authority (EFSA) this did not take into account the body mass of the individuals used in the reviewed studies which range from less than 10 mg, e.g. *Tetragonisca* and *Paratrigona* species, to over 400 mg, e.g. *Bombus* species. Published data on pesticide toxicity in over 20 bee species were reviewed and extrapolation factors proposed taking into account body mass. Further refinement of extrapolation factors by allometric scaling, which takes into account metabolic rate, allows greater understanding of the real drivers of species differences in sensitivity, such as variability in ADME (absorption, distribution, metabolism and excretion) and target/receptor binding. Even these extrapolation factors may be further refined by using the same methodology across species in side-by-side assays to ensure differences are real. In risk assessment it is important to take into account body mass not only in toxicity but also in exposure. However, the key exposure factor which cannot be predicted from bodyweight is the effects of ecology and behavior of the different species on exposure to a treated crop. This is where further data are required to understand the biology of species associated with agricultural crops and the potential consequences of effects on individuals at the levels of the colony or bee populations. This information will allow the development of appropriate higher-tier refinement of risk assessments and testing strategies rather than extensive additional toxicity testing at Tier 1.

90 Taxonomic relevance of an adverse outcome pathway network considering Apis and non-Apis bees

C. LaLone, USEPA / Mid-Continent Ecology Division

Defining the taxonomic relevance of an adverse outcome pathway (AOP) can aid in extrapolation of knowledge from tested model organism to other non-tested species. Recently, an adverse outcome pathway network was developed describing the linkages from activation of the nicotinic acetylcholine receptor in honey bees to colony death/failure. Typically, AOPs are developed with knowledge about, and empirical data from, a single, or handful, of species with uncertainty as to how broadly such pathway-based knowledge can be extrapolated to other species. In describing this AOP network, which primarily focused on honey bees in its development, we begin to evaluate taxonomic relevance in the context of plausible similarity or differences in key events and key event relationships considering non-Apis bees. This evaluation of taxonomic relevance considers, where possible, structural, functional, and modulatory conservation across species. Therefore, the U.S. Environmental Protection Agency's Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) tool was employed to evaluate structural similarity across bee species where proteins were identified as critical to the KE, demonstrating a high degree of conservation at the molecular initiating event and early KEs in the AOP. Additionally, at each level of biological organization, other non-molecular considerations can be used to inform taxonomic relevance, including as examples thermoregulation or foraging strategies, as well as colony structure (or lack there-of) and should therefore be described as a component of the evaluation. Initial efforts in defining the taxonomic domain of applicability relative to chemical stressor perturbation of this AOP network may provide useful insights for protecting or monitoring for adverse effects in non-Apis bees. The contents of this presentation neither constitute nor necessarily reflect USEPA policy.

91 Summary of an international workshop on pesticide exposure assessment for non-Apis bees

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Currently, the honey bee (*Apis mellifera*) is used as a surrogate to evaluate the risk of pesticides to all bee species. However, there is uncertainty regarding the extent to which honey bees can serve as surrogates for solitary bees, bumble bees and stingless bees considering their differences in life history traits (e.g., feeding, sociality, flight/activity season, nesting materials, behavior, etc.). Lack of basic knowledge of non-*Apis* bee exposure scenarios has been one of the biggest challenges in determining whether honey bees are good surrogates. As a result of a tripartite effort between regulatory agencies, academia and agrochemical industry, an international workshop was organized in Washington D.C. on 10th-12th January 2017. Forty bee researchers and risk assessors from ten different countries gathered to specifically discuss the latest state of science on pesticides exposure to non-*Apis* bees and to determine how well the honey bee exposure estimates used by different Regulatory Agencies may or not cover other bee species. After three day discussions there was a general consensus that the current honey bee exposure assessment paradigm is highly conservative. However, several data gaps were identified that hindered the full quantification of exposure to non-*Apis* bees, especially when these bees are exposed via nesting materials such as soil (e.g., blue orchard bees; *Osmia* spp., alkali bees; *Nomia* spp.), leaves (e.g., leafcutter bees, *Megachile rotundata*) or a combination of soil and leaves (e.g., stingless bees; *Meliponini* spp.). Basic conceptual models and preliminary exposure equations were discussed that could help to address quantification of these exposure routes in future and, subsequently, would allow comparison with honey bee exposure estimates. Workshop outcomes along with a list of critical research needs identified for quantification of non-*Apis* bee exposure routes will be available in the form of workshop proceedings and a series of peer review journal manuscripts.

92 Exploring Routes of Pesticide Exposure Risks to Solitary Bees

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Solitary bees experience the environment and its chemical contaminants in some circumstances unlike honey bees experience them. In order to understand the risk of pesticide exposure for solitary bee pollinators, exposure risks that may not be addressed in current assessments using only honey bees must be considered. We illustrate examples of life histories for managed solitary bees that contrast with social bee life histories. Although reported levels of pesticide exposure and toxicity for solitary bees are sparse, some studies have shown that they differ according to bee sociality, seasonality, and nesting behavior. We discuss four important routes of pesticide exposure for solitary, cavity-nesting bees: larval ingestion, adult ingestion, contact, and transovarial transmission.

93 Megachile rotundata: A potential model for non-Apis bee risk assessment

A. Frewin, A. Gradish, G. Ansell, C. Scott-Dupree, University of Guelph / School of Environmental Sciences

In recent years, public and scientific concern has grown regarding the negative impacts pesticides may have on non-*Apis* bee species, partially because current pesticide risk assessments for bees are conducted exclusively on the European honey bee (*Apis mellifera*). Due to fundamental differences in their biology and life-history, it is debatable whether honey bees are an appropriate surrogate species for estimating the risk pesticides pose to solitary bees. To address these concerns, we have started developing semi-field risk assessment methods for use with the alfalfa leafcutting bee (*Megachile rotundata*). The alfalfa leafcutting bee is managed in North America as a pollinator, primarily for alfalfa and canola seed production. As such, its biology is relatively well understood, it is commercial available in North America, and relatively more ecotoxicology data for this species exists compared to other solitary bees. These attributes make the alfalfa leafcutting bee an ideal candidate to serve as the surrogate species for North American solitary bee risk assessments. Our research has involved examining the nesting biology, behaviour, and reproduction of the alfalfa leafcutting bee in small field enclosures and has focused on determining appropriate surrogate crops and release rate combinations for semi-field studies. In addition, we are attempting to determine application rates of toxic reference standards for use as positive controls within this semi-field experimental system. In this presentation, we will discuss method development and describe our experience working with the alfalfa leafcutting bee in the context of semi-field experiments.

94 Development of a semi-field method for use in pesticide risk assessments with Bombus impatiens

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Bumble bees (*Bombus* spp.) are important wild and managed pollinators, and, as such, there is now interest in including data on bumble bees in the risk assessment process for pesticide registration. While standardized, tiered pesticide risk assessment protocols exist for honey bees, these protocols cannot be used for bumble bees because of their pronounced differences in life history and behaviour. Thus, to incorporate bumble bees into the regulatory process, corresponding methods for bumble bees must be development and validated. We conducted a series of studies over 3 years aimed at contributing to the development of a semi-field (Tier II) method for assessing the risk of pesticides to *Bombus impatiens* Cresson, the species that will serve as a surrogate for bumble bee pesticide risk assessments in North America. In 2015, we assessed red clover, purple tansy, and buckwheat as potential surrogate plants for use in semi-field studies with *B. impatiens*. During semi-field studies, bees are exposed to test pesticides while foraging from a treated, flowering surrogate plant; surrogate plants must therefore be attractive to bumble bees to ensure exposure to the pesticide and nutritious enough to sustain colonies for the duration of the test period. We compared *B. impatiens* foraging activity and colony development on all three plant types under semi-field conditions and concluded that buckwheat is an optimal surrogate plant. In 2016 and 2017, our studies focussed on identifying a potential toxic reference standard(s) for use in semi-field studies with *B. impatiens*. Plots of flowering buckwheat were sprayed with dimethoate or diflubenzuron at various concentrations, and one colony was confined to each treated plot for 2 weeks. We compared foraging activity and colony development between insecticides and concentrations, and, based on these results, we provide recommendations for insecticides and application rates that are suitable for use as toxic reference standards with *B. impatiens*. Finally, drawing from the results of all three of our studies, we review trends we have observed in *B. impatiens* behaviour and development under our experimental conditions, discuss the advantages and limitations of our semi-field study design, and provide suggestions for further research. We also highlight differences between our results and results obtained from similar studies with other bumble bee species and stress the necessity of species-specific method development.

95 Field-Level Exposure of Bumble Bees to Fungicides in a Cherry Orchard

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Bumble bees (*Bombus*) are important pollinators of agricultural crops and wild plants. Populations of some species are declining in North America and agricultural chemicals are a possible cause. Fungicides are not thought to be directly toxic to bumble bees, but little is known about sublethal effects. This study evaluates bumble bee exposure to fungicides by quantifying concentrations of boscalid and pyraclostrobin in nectar and pollen collected by lab-reared hives of *Bombus huntii* (Hunt bumble bee) deployed in a commercial cherry orchard in the spring of 2016. One group of seven hives was placed next to a 5-acre orchard block to be sprayed and a control group of seven hives was placed 400 meters away. A fungicide formulation containing boscalid (25.2%) and pyraclostrobin (12.8%) was applied one time at the recommended label rate. Nectar and pollen were collected daily, beginning two days before spray application and continuing for thirteen days. Stored nectar was removed from each hive using a disposable pipette. A pollen trap at the entrance of each hive removed pollen from the foragers as they re-entered the hive. Nectar samples were extracted with acetonitrile using QuEChERS methodology and co-extracted matrix interferences were removed using dispersive solid-phase extraction with primary-secondary amine, C-18 and magnesium sulfate. Pollen samples were extracted with ethyl acetate and did not require further cleanup. Both types of samples were analyzed by GC/MS/MS for boscalid and pyraclostrobin. In the nectar and pollen collected, boscalid and pyraclostrobin concentrations had the same ratio as the pesticide application, approximately 2:1. Nectar concentrations varied spatially by hive and temporally. The highest concentrations occurred 1 or 2 days after spraying: up to 251 ng/mL boscalid and 137 ng/mL pyraclostrobin. The primary and secondary sources of pollen were identified though microscopic visual examination of each bee corbicular load. Pollen from cherries contained the highest concentrations of the fungicides. Control hives and samples collected from treated hives before spraying did not have any pesticides detected in their nectar or pollen. Future work will focus on understanding the effects of these field-realistic doses of boscalid and pyraclostrobin on the long-term development of bumble bee colonies and, specifically, the interactions of these fungicides with important bumble bee pathogens.

96 Evaluating the Potential for Bumble Bee Micro-colonies to Inform Risk Assessment

D. Lehmann, USEPA / ORD NHEERL

Pollinators provide critical ecological services essential to maintaining our food supply and valued natural habitats. Multiple environmental stressors impact the health of managed and native bees in the US and abroad. To satisfy U.S. Environmental Protection Agency (USEPA) requirements, new pesticides are subject to a risk assessment process to identify potential adverse effects on honey bees [*Apis mellifera*] and other pollinators. The National Strategy for Promoting the Health of Honey Bees and Other Pollinators and the 2012 USEPA White Paper describing the conceptual framework for assessing risks of pesticides to bees discussed uncertainties related to assessing exposure and effects from individual pesticides and combinations of pesticides. For example, although there are more than 4,000 distinct species of bees in the US, the honey bee serves as the model organism for USEPA-required risk assessment; in the absence of other data, findings in honey bees are extrapolated to all other species of bees. Limited research is available to evaluate the validity of such extrapolation across bee species. To further address this uncertainty, the USEPA is engaged in the identification and implementation of tests for assessing the impact of pesticides on bumble bee (*Bombus impatiens*) micro-colonies. Bumble bee micro-colonies are formed when a small number of newly emerged worker bees are confined to a queenless environment. Under these conditions, one of the workers assumes a dominant position and begins laying unfertilized eggs that eventually give rise to males (drones). Using this model, pesticide exposure effect studies can be conducted while preserving elements of colony dynamics under well-controlled laboratory conditions. Progression from egg to adult bee can be monitored, allowing for assessment of both lethal and sublethal effects of pesticides. This presentation will discuss efforts to establish a bumble bee micro-colony protocol for use with *B. impatiens* and to investigate the feasibility and potential utility of this model for risk assessment. This abstract does not represent USEPA policy.

In Vitro to in Vivo Extrapolations – Advances and Applications for Risk Assessment

97 In-vitro Biodescriptors in in-silico QSAR Improve the in-vivo Phenotypic Toxicity Prediction Accuracy

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Traditional in silico Quantitative Structure Activity Relationship (QSAR)-based toxicity prediction is susceptible to several limitations, including limited applicability domain with regards to chemical structures, possible inclusion of uninterpretable descriptors, and often lack of mechanistic interpretation of the model. With the increased availability of in vitro

high-throughput screening (HTS) based chemical toxicity data, linking the in vitro HTS assay to the in vivo toxicity endpoint, in line with the adverse outcome pathway, would help to overcome these limitations. In this study, we have explored the bioassay based Quantitative Biological Activity Relationship (QBAR) model, with in vitro HTS-assay as the biological descriptors, to predict in vivo toxicity. The study compares the predictive efficacy of the physical-chemical properties based QSAR model, bioassay based QBAR model, and combined physical-chemical properties and bioassay based Quantitative Structure and Biological Activity Relationship (QSBAR) model. Two separate case studies have been conducted including cytotoxicity (*E. coli* EC_{50}) prediction from *E. coli*-based transcriptomic assay, and genotoxicity (in vivo carcinogenicity) prediction from yeast-based proteomic assay. For the cytotoxicity prediction, the bioassay assisted QBAR models perform better (higher R^2) compared to the conventional physical-chemical based QSAR model or the QSBAR models with both physical-chemical and biological descriptors. Both QBAR and QSBAR models are constructed with biodescriptors derived and quantified at three biologically relevant levels— individual biomarker, specific pathway, and cellular level. Among the three levels, the QBAR model at the pathway level yields the best prediction with the highest R^2 (0.75), followed by the biomarker level model (R^2=0.67). The second case study for in vivo carcinogenicity prediction reveals that, the QBAR model at the pathway level (accuracy=85%, sensitivity=92%, and specificity=71%) outperform the QBAR models with biodescriptors at biomarker or cellular levels, QSAR, and QSBAR models. Though the accuracy of physical-chemical based QSAR model is high (75%), it is not highly informative because of its low specificity (29%). The current study suggests that, bioassay-based QBAR model improves the predictive power of the in silico toxicity prediction and facilitates effective chemical hazard identification and risk monitoring.

98 In vitro assessment of pH-dependent uptake and toxicity of ionizable organic chemicals in fish

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Uptake and effects of ionizable organic chemicals in fish can significantly differ as a function of ambient pH. These differences are driven by the rate of passive diffusion of the uncharged species across the gill epithelium, which is considerably greater than that of charged species. Consequently, the flux of chemicals will peak at different pH values depending on their acid dissociation constants. Here, we propose a rapid in vitro screening method to assess the pH-dependent uptake of ionizable organic chemicals, specifically weak acids, at the fish gill. To this end, the permanent rainbow trout gill cell line RTgill-W1 was grown in transwell tissue culture inserts for two to three weeks, and allowed to establish tight monolayers as characterized by stable transepithelial electrical resistance (TEER). After acclimatization to the reduced complexity exposure medium L15/ex at pH 6.0, 7.4, or 8.5, the permeation of chemicals from apical to basal transwell chambers was determined during a 24-h exposure period by means of liquid chromatography with high resolution mass spectrometry (LC-HRMS). The neutral red retention assay was conducted prior to exposures to exclude interference of cytotoxicity with the measurements. The assay was then used to investigate individual model chemicals (chlorophenols, carboxylic acids) and a technical mixture (nonylphenol) that had been previously shown to cause pH-dependent toxicity. To explore the applicability domain of the assay, we chose to investigate the pH-dependent permeation of chemicals present in acidic, neutral, and basic fractions, as well as reconstituted total extracts of oil sands process-affected water (OSPW). The acute toxicity of OSPWs has been shown to be mostly associated with the acidic fraction, specifically naphthenic acids. We observed a substantial pH-dependency of the cytotoxicity and permeability of weak acids, as well as the acidic fraction and reconstituted total extract of OSPW. We conclude that our in vitro assay can be used to screen for pH-dependent uptake and toxicity of ionizable organic chemicals in fish. It is intended to validate the test for application to prediction of pH-dependent uptake and effects in vivo. Thus, it may be a highly valuable tool for in vitro-in vivo extrapolation, and prioritization of chemicals in non-target chemical screenings.

99 Simulating the toxicokinetics of organic contaminants in in vitro test systems: When do they matter?

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The role of in vitro toxicity testing in the hazard and risk assessment of organic chemicals is expanding and the shift towards these approaches will only increase in the future. In most cases, dose-response relationships from in vitro toxicity tests are reported using nominal concentrations in the test medium despite the known challenges such data introduce for comparing results across different test conditions and between different chemicals. To address some of these challenges, various modeling approaches have been proposed in the literature which can be used to relate nominal concentrations to freely-dissolved concentrations in the medium, concentrations in the cells/tissue, concentrations in the membrane of the cells and chemical activity. Typically, these modeling approaches are based on equilibrium partitioning and therefore cannot address uptake kinetics into cells/tissue, cell growth/division and the potential for degradation in the test system. The main objective of this study was to develop and apply a mass balance model for simulating the toxicokinetics of organic contaminants in in vitro test systems accounting for process such as facilitated transport, growth dilution, and degradation/biotransformation in cells. The toxicokinetic model is applied to a set of hypothetical neutral organic chemicals and then the results compared with a previously developed in vitro mass balance modeling tool based solely on equilibrium partitioning. For relatively persistent chemicals (or in cells/tissue with limited metabolic competency), the simulated mass distribution using the toxicokinetic model is similar to the equilibrium partitioning model output for test durations greater than 24-48 h. In such cases, the simpler modeling approach is deemed sufficient for translating nominal concentrations to more meaningful/reliable toxicity metrics for IVIVE (e.g., C_{FREE} or C_{CELL}). As the rate constants for processes that drive the test system away from equilibrium increase, the discrepancy between the toxicokinetic model output and the equilibrium partitioning model output increases. The discrepancies are a function of physical-chemical and test system properties and demonstrate the need to further develop, evaluate and apply modeling tools to facilitate the interpretation of vitro toxicity test data for hazard and risk assessment.

100 Inclusion of gastrointestinal biotransformation in in vitro to in vivo extrapolation models for bioaccumulation assessment

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An improved understanding of chemical biotransformation in fish has been identified as a critical requirement in the environmental assessment of commercial chemicals. When a chemical is biotransformed, its potential to bioaccumulate in the organism is reduced. Hepatic in vitro biotransformation tests, in combination with in vitro-in vivo extrapolation (IVIVE) and bioaccumulation modeling, is one initiative to complement regulatory bioaccumulation assessment. While hepatic biotransformation assays are useful in chemical bioaccumulation assessment, the gastrointestinal tract (GIT) may contribute substantially to the elimination of hydrophobic environmental contaminants from fish. The diet is often the primary route of exposure for such compounds, yet the influence of intestinal metabolism on the fate of these chemicals is generally overlooked. Standardized methods for measuring intestinal metabolism in fish and incorporating this information into predictive models for chemical bioaccumulation do not currently exist. In this study we measured in vitro biotransformation rates of hydrophobic chemicals (log K_{OW} >4) in rainbow trout hepatic and GIT in vitro systems. Selected chemicals included two organic sunscreen agents (ethylhexyl trimethoxycinnamate and octocrylene) and two PAHs (pyrene and benzo(a)pyrene) as the rates obtained in the GIT and liver in vitro systems could be compared to previously collected in vivo data. Phase I and II enzyme assays were also conducted to compare GIT and liver activities. Additionally, we developed a set of GIT scaling factors to expand a current physiologically based IVIVE model to consider both hepatic and GIT biotransformation. The expanded IVIVE model appears to provide better estimates of in vivo biotransformation potential for chemicals that undergo significant intestinal metabolism. The results also indicate that current hepatic IVIVE approaches may underestimate in vivo biotransformation rates for chemicals that are subject to significant extrahepatic metabolism. Thus, knowledge of intestinal metabolism may improve in vitro estimated biotransformation rate constants.

101 Comparing Effect of Chemicals on Gene Expression Using 3 Chicken Toxicity Models: Hepatocyte, Liver Slice Culture and In Ovo Injected Embryonic Liver

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Seminal reports in the last decade have emphasized that toxicity testing in the traditional sense based on whole animal models is broken, and have brought the need for a paradigm shift to the forefront. This realization, and the potential for lower costs, reduced use of animals, increased efficiency and scalability to high throughput systems have contributed to an increasing interest in the development of alternative methods in environmental toxicity testing. While such models have been developed to greater degrees for mammalian and aquatic species, few have been well established for avian toxicology. Some common avian models include primary cell culture and *in ovo* injected embryo studies. Among the in vitro approaches being explored currently, tissue slices show great promise. Fish and rat liver slices have been successfully used to measure endocrine and metabolic endpoints at molecular and biochemical levels. In oviparous species, the liver is of particular interest as it plays a role in metabolic as well as reproductive function – as part of the estrogenic pathway, it is the site of vitellogenesis which is a precursor to egg yolk production. However, exposure-response profiles have not yet been compared across liver slice cultures and other models. This comparison can be performed by using well established gene expression assays as endpoints that can be measured in all three approaches. The objective of this study is to compare the exposure-response profiles of three toxicological models: liver slice and hepatocyte culture from day 19 chicken embryos and *in ovo* injected day 19 chicken embryos. Cells and tissues were exposed to three chemicals: 17β estradiol, 17β trenbolone, and 2,3,7,8-tetrachlorodibenzodioxin (TCDD) at three concentrations. A Qiagen RT^2 384-well qPCR array was custom designed with 7 genes in reproductive and metabolic pathways including vitellogenin, ApoVLDL, CYP1A4, insulin growth factor 1, hydroxysteroid dehydrogenase, stearoyl-CoA desaturase, and aromatase. Preliminary results show that CYP1A4 expression was upregulated by 91.8 ± 15.6, 120.4 ± 12.5 and 111.6 ± 23.5 in 1nM, 10nM and 100nM TCDD dosed samples respectively, indicating that the slice cultures are metabolically functional. Studying mechanisms of actions at the molecular level can help in understanding effects of contaminants at organismal levels, and in vitro slice culture models could serve as a valuable screening tool to help prioritize chemicals for animal studies.

102 Cross species comparative analysis of in vitro assays supporting IVIVE for the EDSP

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The US Environmental Protection Agency (EPA) established the Endocrine Disruptor Screening Program (EDSP) to determine whether certain substances may have an effect in humans or wildlife that disrupt the estrogen, androgen or thyroid axes. EPA developed an 11 assay Tier 1 screening battery, which includes 6 in vivo assays in rodent, fish and amphibian test systems, to help identify chemicals which can potentially interact with the endocrine system. The EDSP is now utilizing validated computational and high throughput (HTP) models to more efficiently and rapidly assess chemical bioactivity as a part of a larger prioritization effort for endocrine disruption screening. To support this pivot, the EPA is investigating in vitro to in vivo extrapolations (IVIVE) to link bioactive concentrations generated from HTP in vitro assaysto estimate in vivo oral doses or aquatic concentrations and predict endpoints traditionally measured using animal assays. EPA is conducting in vitro assays, plasma protein binding and metabolic clearance in cryopreserved hepatocytes, to collect input chemical and species specific data for physiologically-based toxicokinetic (PBTK) models necessary for IVIVE. Once properly validated, IVIVE may replace some whole animal endpoints or tests (e.g., EDSP Tier 1 assays, fish bioaccumulation predictions, or other short-term overt toxicity assays). IVIVE coupled with species extrapolation of mammalian test data would allow comparison of relative species internal doses which will prioritize data gaps for wildlife testing and assessment. A total of 55 chemicals are being tested in both in vitro assays using human, rat or trout biological material. Species specific differences in clearance rates are evident in preliminary clearance data. Preliminary plasma protein binding data indicate that triflumizole, 3,3',5,5'-tetrabromobisphenol A, 4-*n*-nonylphenol, and triclocarban were each 99% bound (at 10 µM) in plasma of all three species. For propanolol at 10 µM, plasma protein binding was similar for rat and human (ca 70-75% bound), while for trout it was 93%, indicating that there can be species differences in plasma protein binding. In general, the fraction unbound is similar across the concentration range 0.1 to 1 µM within a species. This abstract does not necessarily represent USEPA policy.

103 Universal toxicokinetic constants for metabolic decomposition and chemical uptake predict bioconcentration factor data for diverse chemical exposures

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A great challenge for in vitro to in vivo extrapolation (IVIVE) is to reliably predict the physical state of organisms exposed to chemicals in the environment from in vitro exposure assay data. Although physiologically based toxicokinetic and the associated reverse toxicokinetic (rTK) modeling approaches promise to bridge inexpensive in vitro screening data with relatively costly in vivo effect data, they are encumbered by a need for redesign or re-parameterization when applied to different tissues or chemicals. We demonstrate how these problems can be overcome by using rTK models developed for the adult zebrafish (*Danio rerio*) to predict bioconcentration factors (BCF) for a range of chemicals without retraining the model. Particle swarm optimizations correlated the rates of aquatic respiration and the metabolism specific to individual chemicals, wherein the median correlation reveals a "universal" value that permits BCF predictions with high statistical agreement to diverse chemical data. Results suggest that chemical uptake into arterial blood is mechanistically limited by transport across respiratory gas-exchange membranes. Optimized rates aquatic respiration rates are smaller than literature values, suggesting a shift in emphasis to a more detailed molecular understanding of aquatic respiration. Although these models work well for predicting adult zebrafish BCFs, they are generally incapable of predicting BCFs measured in other fish species. In contrast, we find the adult zebrafish models to be modest predictors of BCFs measured in zebrafish embryos, opening the door to the possibility of using in vitro embryo assays to evaluate the exposed adult zebrafish state.

104 Fish embryo testing (FET) with the estuarine eastern blue spot goby (Pseudogobius sp.): Method development, sensitivity, and current unknowns

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The use of fish embryo testing (FET) is strongly being encouraged to replace acute and chronic toxicity testing with juvenile fish. Much of the work that has been conducted using FET has been to evaluate chemical safety and effluents with guidance proposed and developed by OECD in 2006 and ISO in 2007. The current literature typically uses one of three model freshwater fish species, zebrafish (*Danio rerio*), the Japanese medaka (*Oryzias latipes*) or the fathead minnow (*Pimephales promelas*). These species are commonly used since information pertaining to physiology, embryonic development, and sensitivity are abundant. However, none of these fishes occur naturally in Australia, and therefore lack environmental realism (in an Australian context). Therefore, for Australian environmental monitoring and ecotoxicological research there is a need to establish equivalent tests in locally relevant species (freshwater, estuarine and marine). This presentation will showcase some of the fish embryo endpoints currently being assessed (heartbeat/min, cumulative hatch rates, survival, size at hatch) in the eastern blue spot goby (*Pseudogobius sp.*) a small, estuarine species native to southeastern Australia. Specifically, this presentation will discuss some of the strengths and limitations of this embryo assay as it pertains to culturing, testing setup and methodology (for both water and sediments) as well as the species sensitivity and environmental realism/relevance.

The Science of Safer Chemistry – Researchers, Retailers and Beyond

105 Best Practices for Alternatives Assessment: Case Studies from Northwest Green Chemistry

A. Nestler, L.G. Heine, Northwest Green Chemistry

In order to design and select products that will stand the test of time, a neutral and comprehensive assessment of available and emerging alternatives is necessary. An Alternatives Assessment (AA), as defined by the Interstate Chemicals Clearinghouse (IC2), is a process for identifying and comparing potential chemical and non-chemical alternatives that can be used as substitutes to replace chemicals or technologies of high concern. As an independent non-profit, Northwest Green Chemistry (NGC) can perform assessments that complement government regulation, inform procurement policies, allow for increased communications with industry leaders, and function as drivers for the adoption of safer chemicals. In this presentation, NGC will share our methodology for whole product assessment that goes beyond single chemical substitution, provide lessons learned from a number of efforts and make recommendations for linking AA results to product design. Highlights include scoping methodology, stakeholder engagement, framework flexibility, whole-product & life-cycle evaluations, AA as a driver for product reformulation, and the importance of having a plan B. One example that will be discussed is the Washington State Antifouling Boat Paint Alternatives Assessment which was performed due to state legislation phasing out copper-based antifouling boat paints for recreational users. Two other examples that will be discussed are in-progress AAs on anticorrosion bridge coatings (a project partnering with local and state governments that addresses air and water quality concerns) and alternatives to fluorinated additives to provide grease-proofing in single use food takeout containers. NGC is also working to link AA with product design. We have partnered with Autodesk, Berkeley Center for Green Chemistry and the Cradle to Cradle Products Innovation Institute to further develop a framework to inform material and product design for the circular economy for us in additive manufacturing (3D printing). NGC will share our experiences and lessons learned from these projects in order to foster discussion on AA best practices.

106 Safer chemical criteria in ecolabels: Driving adoption of safer chemistry through market access requirements

C.D. Robertson, Hewlett-Packard / Sustainability

Alternative assessment and hazard assessment are starting to be incorporated into ecolabels and certification criteria. The U.S. Green Building Council's LEED certification now incorporates hazard assessment into building material requirements. An important ecolabel for the electronics industry is TCO Development's TCO Certified label. TCO has incorporated an accepted substance list for flame retardants based on the GreenScreen for Safer Chemicals. In the United States, EPEAT certification is arguably the most important Green Public Procurement (GPP) requirement that must be met in order to bid on many government purchasing contracts. Safer chemical criteria have been drafted and will likely be included in the next revision of EPEAT for personal computer products. Aspects of these ecolabels will be presented as well as a discussion of their ability to influence real-world adoption of safer chemicals. In the course of developing safer chemical criteria in ecolabels several competing interests are revealed. Stakeholders include manufacturers, suppliers, government agencies, non-governmental organizations (NGOs) and the chemical industry. Additionally, the owners of hazard assessment frameworks, such as the GreenScreen, place conditions on market claims made using their methods. The Business-NGO Working Group (BizNGO) is developing a white paper to help guide the incorporation of the GreenScreen and hazard assessment in ecolabels. Best practices and implementation experience gained through development of ecolabel standards will be shared in this presentation.

107 High Throughput Screening-Level Human Health Risk Assessment for Surveying Safety of Cleaning Product Ingredients: Methods and Results

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The American Cleaning Institute (ACI) conducted screening level risk assessment for cleaning product ingredients in member company products. ACI's approach demonstrates how data mining and conservative risk models can be used to survey ingredient inventories across product sectors, assess risk, and identify opportunities for green engineering. Survey of ingredient listings for 900 member products was performed to identify 588 ingredients relevant to 36 different cleaning product types. Ingredient concentrations in each product type were mined from product sheets, generic formulations, scientific publications, trade literature, and government reports. A range of estimated concentrations were established for each unique ingredient in each product type. Previous studies on habits and practices for each product type were combined with concentration data to populate deterministic exposure models covering direct and indirect dermal, indirect oral, and direct inhalation routes of exposure for intended use. Intake estimates were then calculated for upper and lower bounds of potential exposure. To provide hazard data for each ingredient, a database of over 7000 relevant data points was compiled from hazard studies published via REACH, HPVIS, OECD, HERA, and comparable sources. For some ingredients, existing risk assessments were found which demonstrate ingredient safety. Derived no effects levels (DNELs) were derived from the hazard database using hierarchical prioritization of data sources and types; application of assessment factors; and structure-based read across. These DNELs were used together with aggregated exposure estimates to calculate risk characterization ratios (RCRs) across exposure pathways and product use types. Hazard and exposure modeling used conservative assumptions suitable only for screening level assessment. Risk conclusions were developed using either quantitative assessment via RCRs or qualitative assessment based on findings of previous studies. Of the ingredients assessed, 99% demonstrated either a RCR less than 1 under all scenarios modeled, or a safety finding by a transparent and defensible source using comparable methods. Four ingredients had a RCR of 1 under scenarios using most conservative assumptions; refined models using more realistic assumptions demonstrated RCRs consistently below 1. A small number of ingredients were not assessed because they are proprietary, and safety data is held by the manufacturer.

108 Advancing Alternatives Assessment in Public Policy and Programs: Lessons Learned from North America and the European Union

J.A. Tickner, UMASS Lowell / Community Health and Sustainability; M. Jacobs, University of Massachusetts Lowell / Lowell Center for Sustainable Production

Increasing marketplace attention and consumer demands to substitute chemicals of concern have driven much of the growth in the field of alternatives assessment in recent years. Yet, evidence suggests that government programs and regulatory policies remain significant drivers of substitution efforts globally. How alternatives assessment and substitution are being considered in a broad array of government policies and programs in North America and the European Union will be reviewed based on recent alternatives assessment policy research and analysis studies. These studies involved policy/legal reviews as well as interviews with government officials and chemicals management experts. The analyses reveal that a range of policy incentives and disincentives to use alternatives assessment are needed to support a broad transition to safer chemicals. Policies that simply prohibit or place conditions on chemicals of concern without requirements to carefully evaluate alternatives can lead to regrettable substitutions. Innovation in safer chemistry requires both regulatory elements that drive actions to evaluate safer alternatives to chemicals of concern as well as support in the form of incentives, technical assistance and education to adopt those alternatives. These public policy and programmatic elements will be reviewed, with an emphasis on the need to connect alternatives assessment and substitution efforts to green chemistry innovations and the need to more broadly advance alternatives assessment capacity within government agencies.

109 Using 21st Century Science in Toxicology Evaluations: DoD Example

M.S. Johnson, US Army Public Health Center / Toxicology

Proactive assessment of environmental, safety, and occupational health hazards is essential and required in the production and use of new weapon systems and platforms; however, few specific data requirements exist. Additionally, no specific requirements exist for research, development, testing and evaluation (RDT&E) of new technologies. Any effective approach needs to be integrated at the beginning of research when new compounds and formulations are considered, and it is essential that collection of such data be commensurate and proportional with the research level of investment. Current guidelines provide a phased approach that allow for the incorporation of *in silico*, in vitro, and focused in vivo methods for new compounds. Some of these new methods have yet to be validated; however, data can be collected in a relative manner and be used alongside other performance criteria in the assessment of RDT&E programs. Examples will be provided.

110 USEPA Safer Choice: Tools and resources to move towards safer chemistry

L.E. Sweet, USEPA / Safer Choice Program

The focus of the USEPA Safer Choice program is to encourage safer chemistry by differentiating the safest chemicals that can satisfy a functional use within consumer products. Safer Choice is a voluntary program that serves as a component of the USEPA's chemical safety program, which is anchored in the Toxic Substances Control Act (TSCA). Rather than focus on regulatory mandates, the Safer Choice program focuses on the green end of the chemical spectrum, providing certainty for industry seeking chemistry that will help them remain competitive in the long term. The Safer Choice label on qualifying products provides assurance to consumers that a product contains safer chemical ingredients that meet stringent human and environmental health criteria without compromising performance. Safer chemistry appeals to retailers, purchasers, and consumers in the marketplace; thus, the Safer Choice label provides an incentive for the use and manufacture of safer chemicals in consumer products. The success of this voluntary program has largely been due to the support of industry, retailers, NGOs, and consumers. The standard behind the Safer Choice label was developed using a multi-stakeholder process and is aligned with the low concern thresholds under the TSCA New Chemicals Program and Globally Harmonized System of the Classification and Labeling of Chemicals. The Safer Choice labelling program is a source of chemicals for USEPA's Safer Chemical Ingredient List (SCIL), an important resource that assists manufacturers with finding safer, low hazard chemical alternatives. To date, the USEPA Safer Choice program has labelled about 2000 products and listed over 800 chemicals on SCIL. SCIL has applications beyond use in Safer Choice, as the list continues to grow with the addition of 100 new chemicals each year, making the availability of safer chemicals better known in the marketplace, and promoting opportunities for innovation in green chemistry beyond the cleaning industry. Through the Safer Choice program, USEPA is growing the list of safer chemicals available in the marketplace. Collaborative participation of a range of stakeholders makes it possible for this program to help safeguard human and environmental health.

111 Identification of Chemical Candidates for the USEPA's Safer Chemical Ingredients List

M. Kawa, C. Rudisill, L. Morlacci, J.L. Tunkel, SRC, Inc. / Environmental Health Analysis

The USEPA Safer Chemical Program's (SCP) Safer Chemical Ingredients List (SCIL) identifies safer ingredients using robust, transparent and publicly available criteria for several functional use classes including surfactants, preservatives, solvents and chelants. Efforts to further populate USEPA's SCIL with more substances supports product formulators by providing a dynamic palette of safer chemicals. Typically, SCIL is expanded based on stakeholder input, using a top-down approach where SCIL candidate chemicals are obtained mainly/directly from product submissions. The goal of the work presented in this poster was to identify more chemicals that meet SCIL criteria, using a bottom-up approach where candidates were identified from the available chemical inventory. This new approach required design and implementation of a plan for identifying chemicals most likely to meet SCIL criteria, as well as increasing the variety of functional uses on SCIL. Adding new substances to SCIL also contributes to the inclusion of new product sectors by targeting chemicals used in multiple product types. Queries were run to find chemicals with specific functional uses or product sectors from a sample of freely available public data sets. Sources included the USEPA's Chemical and Product categories (CPCat) database, the European Commission's database for information on cosmetic substances and ingredients (CosIng), EWG's Skin Deep® database, and the NIH's Household Products Database. Ideally, chemicals listed in these resources are available on the market and have a demonstrated functional use. The chemical inventory obtained from these sources was curated by removing known PBT and CMR chemicals and collecting chemical structures. The curated set of chemicals were clustered with existing SCIL chemicals using USEPA's

ChemACE software to identify substances that are structurally similar to those chemicals already meeting SCIL criteria. Clusters containing both SCIL and non-SCIL chemicals were chosen for further review. Separately, REACH's ECHA and the Danish QSAR data sets were queried to identify chemicals with experimental or estimated values that would pass SCP criteria. Use information was collected for the chemicals with potential to pass criteria. Candidates from the queries were prioritized and queued for assessment against SCP criteria. This work has resulted in the addition of more than 40 chemicals to the SCIL.

112 Integrating safer chemicals into consumer product materials decision-making: Enabling chemical screening and alternatives assessment "upstream"

J.P. Rinkevich, P. Beattie, C. McLoughlin, SciVera LLC

Product development teams and product designers are demanding improved ways to evaluate materials for products based in underlying chemical health and environmental characteristics. Traditional manual approaches to chemical screening and assessment cannot keep up with the dynamics of changing regulations, short product development cycles, and increased expectations for going beyond basic regulatory compliance toward green chemistry achievement in consumer products and their processes. Moreover, the need for scaled evaluation of thousands of chemicals in tens of thousands of materials for the average consumer products brand makes the traditional, manual approaches cost- and time- prohibitive. Our work over the past several years has focused on meeting these growing needs for chemical and material screening efficiencies in consumer products companies. Most recently we worked to push this capability upstream to raw material suppliers, specifically in textile processing to assist in evaluation of potential human health and environmental/aquatic impacts. Putting this chemical screening capability in front of raw material developers, where the formulation knowledge exists, can enable better decisions during materials and process chemical development. We have recently established a step-wise, automated system to rapidly screen chemicals based on available experimental, authoritative, and modeled/analogous data, leveraging established expertise in data interpretation to deliver actionable alternatives assessment guidance to raw material and process chemicals development professionals. Our presentation will provide an overview of this upstream step-wise process and the underlying automation to make chemical hazard alternatives assessment accessible to decision-makers upstream in the consumer products value chain, which consider the varying level of chemical and toxicology expertise inherent in these organizations. Supporting evidence will include example comparative assessments at the chemical and material level, time required, results achieved, and conclusions drawn from the output.

Measurements and Methods in Environmental Nanotechnology in Aquatic Systems

113 Trophic transfer of silver nanoparticles in freshwater ecosystems - should we be concerned?

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Metal-containing engineered nanoparticles (Me-ENPs) are used in a wide range of products, such as inks, plastics, consumer products, lubricants, electronics and bioactive coatings. Silver (Ag) ENPs are one of the most used Me-ENPs to date, primarily due to its antibacterial effects. When entering the aquatic ecosystems, Ag ENPs will undergo several transformation processes, ultimately resulting in the particles settling out of the water column. This will likely result in an increased concentration of ENPs in the sediment. In fact, predicted environmental concentrations of Ag ENPs in Danish and European freshwater ecosystems range from a few ng/L in surface waters and up to >1000µg/kg in sediments. Several studies have shown Ag ENPs to be toxic, bioaccumulative and harmful to aquatic biota within these concentration ranges. However, research on potential trophic transfer of Ag ENPs is limited. To study trophic transfer from the abiotic sediment compartment, to the first links in the freshwater food chain, we designed a relatively simple model system: First, we exposed the sediment dwelling oligochaete *Tubifex tubifex* to sediment amended with Ag ENPs for 23 days. Worms were then converted into food packages, by homogenizing them in artificial freshwater (ISO 203), mixing the homogenate with alginate and creating droplets of the mixture in a $CaCl_2$ solution. Secondly, we fed the worm-packages to the freshwater fish, *Danio rerio*. Fish were exposed to this Ag ENP contaminated food source for up to 14 days, followed by up to 14 days of elimination (i.e., fed uncontaminated food). Results showed that Ag was present in fish after exposure, and that the silver was mainly found in the intestinal and gut regions. This highlights the possibility of Ag ENPs to be trophically transferred from the sediment to the food web. This could have adverse effects for our aquatic environments, as zebrafish are natural prey to many higher organisms such as knife- and catfish. In addition, our results show that sediment is not only a sink, but also a source of ENPs in freshwater ecosystems. Thus, future studies should include the sediment compartment when investigating the fate of Me-ENPs, and incorporate more complex food web setups to assess the long-term effects of Me-ENPs on our aquatic ecosystems.

114 Linking Chemical Transformations of Silver Nanoparticles in the Extracellular and Intracellular Environment to their Bioavailability and Toxicity

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Determining the exposure scenarios that enhance or reduce metal nanoparticle uptake, bio-reactivity, and toxicity is often difficult due to the lack of appropriate biological models. To allow for such investigations at the cellular level, a model of the fish intestine derived from rainbow trout (*Oncorhynchus mykiss*), the RTgutGC cell line, was used. Once inside the cell, the bio-reactivity and toxicity of nanoparticles depends on their chemical transformation, a process which is currently poorly understood. While it is possible to determine dissolved metal speciation outside the cell using simplified cell culture media and chemical equilibrium models, intracellular metal speciation is more difficult to ascertain. Accurate evaluation of intracellular metal speciation requires a technique that allows for in situ measurement, such as X-ray absorption spectroscopy (XAS). In this study RTgutGC cells were exposed to silver nitrate ($AgNO_3$) and citrated coated silver nanoparticles (cit-AgNP) in a medium of well-defined composition (L-15/ex) which made possible the determination of the dissolved silver species using visual MINTEQ. We have used XAS to track the intracellular metal speciation of silver in RTgutGC following exposure to $AgNO_3$ or cit-AgNP. Cell monolayers seeded on transwell inserts were exposed to 1 µM $AgNO_3$ and 10 µM (1.07 mg/L) cit-AgNP for 1, 24 and 72 hours in L-15/ex. Previous experiments showed that such exposures were not-toxic. Intracellular silver speciation changed over time in both exposure series. After 1 hour, intracellular silver speciation is dominated by chloride complexation in both exposures. This data supports the hypothesis that silver enters the cell as a silver chloride complex. After 24 and 72 hours of exposure to AgNP, 8 and 5% of silver were complexed to cysteine respectively, whereas the remaining silver was AgNP. In cells exposed to $AgNO_3$ for 72 hours, 97% Ag is complexed to cysteine. Our previous data showed a significant increase in metallothionein mRNA levels which would explain the formation of Ag-cysteine complexes. As mentioned earlier, $AgNO_3$ and AgNP were not toxic, suggesting that at this concentration metallothionein is able to scavenge most dissolved silver in the cell. These results provide new insight into our understanding of Ag and AgNP uptake and toxicity in fish intestinal cells. Future studies will focus on their impact on the homeostasis of essential metals.

115 Mass spectra-based metabolomics of Arabidopsis thaliana exposed to silver nanoparticles

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Silver nanoparticles (AgNPs) are one of the most commonly used engineered nanomaterials in consumer products due to their antimicrobial properties. AgNPs can be introduced into the environment, when products containing AgNPs are disposed of in landfills or leach out from products during washing, eventually reaching wastewater treatment plants, and accumulates in biosolids. Consequently, the land-application of biosolids to fertilize fields or usage of reclaimed water can lead to the unintentional exposure of AgNPs to different biological organisms. In this study the impact of citrate and polyvinylpyrolidone (PVP) coated silver nanoparticles on a model plant, *Arabidopsis thaliana* was investigated using hydroponic cultures, with the objective of providing quantitative and qualitative descriptors of the uptake process. Analysis of AgNPs was performed using Inductively Couple Plasma Mass Spectrometry (ICP-MS) for total silver analysis, and metabolite profiling was achieved by Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry (LC-QToF-MS). Results show silver translocation from treated *Arabidopsis thaliana* roots to leaves and flowering shoots in all treatments. Furthermore, metabolomics results indicate up and down regulated species in silver treated *Arabidopsis*. The identification of species that differentiate during AgNP treatment are to be determined. Correlating the silver concentration in *Arabidopsis thaliana* and changes in metabolite profile provides insight on the mechanism and interactions between nanomaterials and biological organisms. This is of importance for understanding the potential risks associated with AgNPs released into the environment.

116 Understanding comparative toxicity of transformed ZnO nanoparticles through uptake dynamics and transcriptional responses in Hyalella azteca

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As ZnO nanoparticles (NPs) make their way into the aquatic environment, water chemistry will influence their fate, transport, and extent of their dissolution. Although the fate and transport of ZnO NPs is complex, sedimentation is expected in most surface water environments, which will put benthic organisms, including the amphipod *Hyalella azteca*, at particular risk. In addition, ZnO NPs undergo a complex set of transformations in the environment, including pH dependent changes in speciation and solubility and transformations into phosphates, sulfides and carbonates. The goal of our research is to understand the relative toxicity and uptake of environmentally transformed ZnO NPs to *H. azteca* and reveal the physicochemical factors and biological effects responsible for the differences in toxicity. *H. azteca* were exposed to ZnSO4, as-synthesized ZnO NPs, and artificially aged ZnO NPs which resulted in three types of particles: 30 nm ZnO-Zn3(PO4)2 core-shell structures, micron scale hopeite-like Zn3(PO4)2*4H2O), and ZnS nano-clusters. Previous studies in our laboratories have shown that ZnO NPs are more toxic to *H. azteca* then Zn ions with phosphate aged NPs showing the highest toxicity. In contrast, sulfide aged particles (ZnS nano-clusters) have greatly reduced toxicity. To clarify the mechanisms responsible for the differences in toxicity, we used the precepts of a kinetic bioaccumulation model to investigate differences in uptake and elimination, as well as transcriptomics to better characterize the different biological responses across the particles. For the biodynamic studies, we exposed *H. azteca* to Zn ions, as-synthesized ZnO NPs, or ZnS nano-clusters in short term feeding experiments and 24h water exposures. Zinc uptake and elimination were traced using Zn isotopically enriched (^{67}Zn or ^{68}Zn). In addition, we performed RNAseq analysis following 96-h exposures to the treatment specific LC25 for each of the transformed ZnO particles and Zn ions. Gene expression analysis will enable us to determine if all four particles exert toxicity through a similar mechanism of action. Together the present studies will provide essential information for risk assessments by increasing our understanding of the processes that govern differential toxicity of NPs.

117 The Interactive Effects of UV Radiation and TiO2 Nanoparticles on the Calanoid Copepod, Acartia tonsa

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Titanium dioxide nanoparticles (n-TiO_2) found in paints, sunscreens, and skin-care products are entering the marine environment through run-offs, product usage and industrial wastewater. The accumulation of nanomaterials in the environment is likely non-uniform, with coastal waters impacted by higher concentrations. TiO_2 (bulk and nano forms) is a photocatalyst, able to produce reactive oxygen species (ROS) that cause deleterious effects in some aquatic organisms. This study investigated the toxicological effects of commercially available n-TiO_2 on the female calanoid copepod, *Acartia tonsa*. Using bulk TiO_2 as a control, we were able to disentangle nano effects from combined nano/photocatalytic effects. Four experimental treatments, a) copepods with n-TiO_2 and food, b) copepods with food only, c) n-TiO_2 with food and d) food only, were exposed to different light conditions (6-hr light, 4-hr light and 0-hr light as a control). Light was supplied by solar lamps that mimic natural light in the visible and UVA/B regions (Solarlux®, EYE Metal Halide System). Initial and final algae concentrations were determined using an electronic particle counter (Coulter Multisizer) to evaluate the effects on copepod ingestion rate (IR). Egg production rate (EPR) was determined after 24 hours of exposure, and egg hatching frequency (HF) was determined at 48 hours. Preliminary results indicate that both n-TiO_2 and light affect IR, EPR and HF of copepods compared to the bulk TiO_2 and 0-hr light controls. This work suggests that nanomaterials under realistic environmental conditions could have organismal and population impacts, and will help to inform future studies on organisms that rely on zooplankton for food (e.g., fish larvae and filter-feeders).

118 Growth-based bacterial viability assay for interference-free and high-throughput nanotoxicity screening

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High-throughput toxicity screening on microorganisms with minimal interference is needed to understand the effect of increasingly complex nanomaterials within ecological systems. Currently, most high-throughput screening assays for bacteria viability rely largely on optical assays based on fluorescence or absorbance. However, as nanomaterials often display conflicting optical properties, interference by nanomaterials in optical assays can be significant. Herein, we developed a high-throughput bacterial viability assay that is largely free of interference. In this assay, bacteria are exposed to various potential toxicants and then diluted into large amount of fresh growth medium. The fraction of viable cells after exposure start propagation in fresh medium, generating growth curves. Bacterial viability can then be quantitatively correlated to the delay of bacterial growth compared to a reference; data analysis was inspired by that in quantitative polymerase chain reactions, where the delay in the amplification curve is correlated to the starting amount of template nucleic acid. The large dilution insures minimal optical interference from the nanomaterial when reading optical density, and the residue left from the exposure mixture after dilution was confirmed not to affect the bacterial re-growth profile. Fast and robust data analysis was achieved by developing computer algorithms carried out in R code. To date, this method has been tested on five bacterial strains, including Gram-positive and Gram-negative

bacteria, showing great potential for general use with culturable bacterial strains. With the increasing diversity of engineered nanomaterials being considered for large-scale use, this high-throughput screening method will facilitate the rapid screening of nanomaterial toxicity and thus inform the risk assessment of nanoparticles in a timely fashion.

119 Competitive adsorption between different PAHs on carbon nanotubes and the impact on adsorbed-PAH bioavailability to P. promelas

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Due to the high adsorption affinity of carbon nanotubes (CNTs) for polycyclic aromatic hydrocarbons (PAHs), these materials are have been considered both an ideal substrate for pollution remediation and a potential risk as a "contaminant carrier". Our previous work investigating a suite of PAHs found that bioavailability of CNT-adsorbed PAHs was a function of adsorption behavior and was significantly influenced by PAHs physical characteristics, particularly molecular configuration and size. To date, no study has investigated the bioavailability of mixtures of PAHs adsorbed to CNTs though this is a much more likely scenario to occur environmentally and may elicit different trends in bioavailability than previously observed in single-solute scenarios. The goal of the present research was to investigate the adsorption behavior of PAH mixtures on CNTs and how this relates to the resulting bioavailability of the PAHs present. Bi-solute adsorption isotherms of chemically similar PAHs by CNTs were established in conjunction with quantifying the bioavailability of the two competing CNT-adsorbed PAHs to *Pimephales promelas* (fathead minnow) using bile analysis via HPLC fluorescence detection. Ratio comparison of the fish response to each PAH present in single-solute and bi-solute treatments, with and without CNTs provide insight to the interaction occurring between the PAHs at the CNT surface. Results showed, that chemically similar PAHs, pyrene and fluoranthene, were equally competitive in the adsorption studies, although the corresponding bioavailability assays showed a significant increase in fluoranthene bioavailability when pyrene was also present. In contrast, angular phenanthrene was more bioavailable than its linear isoform, anthracene, when adsorbed to CNTs in single-solute solutions, but in mixture bioavailability assays there was no observed difference. These two bi-solute mixtures suggest that competition is less influenced by structure (i.e. linear vs angular), but more by the makeup of the compound where fully aromatic PAHs will outcompete those containing a non-aromatic ring. Further, the data indicates that interactions within an organism can causes changes in competitive behavior between compounds even when physical adsorption appears similar in aquatic systems. Results of this work help to provide insight into the impact of CNTs on PAH bioavailability in more realistic scenarios.

120 Method Selection Framework for the Quanification of Nanocarbon

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There is significant uncertainty regarding the impact of nano carbon (nanotubes, graphene, fullerene, etc.) on human health and the environment, and the ability to accurately determine exposure levels is critical to filling in these knowledge gaps. Given the various analytical challenges associated with the detection and quantification of nano carbon, it is unlikely that a single method or technique will prove effective for all forms of nano carbon or in all exposure scenarios; the optimal approach will likely depend on the nature of the material being measured, its concentration, and the matrix in which it is contained. In this work, a decision framework will be presented that assists a user in determining which analytical methods are best-suited for a given sample.

Flame Retardants – Sources, Environmental Behavior, Wildlife and Human Exposure, and Effects Implications

121 Influence of aqueous conditions on the leaching of diverse organic flame retardants from microplastics

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The volume of plastics used and released to the environment is increasing every year. Large amounts have thus amassed therein. Flame retardants (FRs) and other additives are present at % levels in many polymer products. Hence, plastics are an important reservoir and source of FRs to both indoor and outdoor environments. Historically, additives have been some of the more important emerging contaminants. Organic FRs include brominated, chlorinated and non-halogenated chemicals. FRs exhibit a range of volatilities and water solubilities. Plastics show varying vulnerabilities to weathering, primarily abrasion and UV degradation. During use, and after discard, polymers fragment into microplastics, which are of increasing concern in indoor dust, surface and wastewaters, as well as sewage sludge. In lab experiments,UV irradiation affected surface areas of polymers (polyurethane, polyethylene, polystyrene, PVC, ABS) differently. We subsequently examined leaching of diverse FRs from polyurethane foam and HBCD from expanded polystyrene. For polyurethane, higher water temperature (4-40^{0}C) increased leaching of hydrophobic brominated (BFRs), but not the more soluble phosphate FRs. DOC (0-30 mg/L) in the form of Aldrich humic acid had a greater effect than temperature treatments, and two synthetic gut fluids enhanced BFR leaching even more. Increased salinity (0-35 psu) decreased leaching modestly, likely due to the salting out effect. Surprisingly, little leaching of HBCD was observed from expanded polystyrene. We conclude that aqueous leaching and subsequent bioavailability of FRs are a function of the properties of the FR, polymer and surrounding aqueous phase. These findings also have ramifications for designing greener plastics, FRs and better wastewater treatment.

122 Methylation of TBBPA by methyl iodide in aqueous system

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Tetrabromobisphenol A (TBBPA) and tetrabromobisphenol A dimethyl ether (TBBPA DME) have been detected in various environmental matrixes. TBBPA DME was regarded as the transformation product of TBBPA since it has no commercial production. The transformation from TBBPA to TBBPA DME has been reported in organisms and plant cells. Methyl iodide (CH_3I), the most abundant organoiodine compounds in natural environment, is a widely studied methyl group donor. The application of CH_3I as fumigant to replace the ozone-depleting fumigant methyl bromide is increasing its anthropogenic input into the environment. CH_3I can methylate Sn^{2+}, As^{3+}, Pb^{2+} and Hg^0 by the oxidative addition mechanism. However, the understanding about the effects of CH_3I on the methylation of organic compounds, such as the phenolic contaminants was very limited. In this study, methylation of TBBPA was investigated under the simulated solar light irradiation and darkness condition. Under light irradiation, tetrabromobisphenol A monomethyl ether (TBBPA MME) were found as the transformation product of TBBPA in 5 min with transformation ratio less than 0.01 %. There was no further methylation product of TBBPA DME in the system. And the TBBPA MME was disappeared after 10 min, indicating the further degradation. In the 15 days' experiment with darkness condition, the methylated products were also detected. The methylation of TBBPA was affected by the pH of the aqueous solution. At pH 1 and 14, only TBBPA MME (without TBBPA DME) was found with the transformation ratio of 0.006 ± 0.001 % and 0.53 ± 0.07 %. At pH 7, both of the TBBPA MME and TBBPA DME were found with the transformation ratios of 0.95 ± 0.09 % and 0.003 ± 0.001 %, respectively. Compared with the darkness, light significantly accelerated the methylation of TBBPA. The results indicated a new potential pathway to generate TBBPA MME and TBBPA DME from TBBPA.

123 Screening of Organophosphate Flame Retardants and Emerging Metabolites in Municipal Sludge from Chinese Wastewater Treatment Plants Nationwide

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Little is known about the occurrences, distributions, sources and potential risks of organophosphate flame retardants (OPFRs) and emerging metabolites in municipal sludge from wastewater treatment plants (WWTPs). Recently, we carried out a nationwide survey to simultaneously determine 11 OPFRs and 6 emerging metabolites in sludge from WWTPs across China. All OPFRs were positively detected and 4 metabolites bis(butoxyethyl) phosphate (BBOEP), bis(2-chloroethyl) phosphate (BCEP), bis(2-chloropropyl) phosphate (BCIPP) and bis(1,3-dichloro-2-propyl) phosphate (BDCIPP) were identified for the first time in sludge samples. The total concentrations of OPFRs and emerging metabolites were in the range of 43.9−2160 ng g^{-1} dw and 17.0−1300 ng g^{-1} dw, respectively, indicating a moderate pollution levels of OPFRs in China compared with the developed countries. A distinguishable geographical trend of higher sludge concentrations of OPFRs and emerging metabolites in East China than Central China and West China can be observed, implying that regional pollution level of OPFRs is associated with economic development level. Significantly positive correlations were found between sludge concentrations of tris(2-butoxyethyl) phosphate (TBOEP) *vs* BBOEP ($R = 0.70$, $p < 0.01$) and triphenyl phosphate (TPHP) *vs* diphenyl phosphate (DPHP) ($R = 0.71$, $p < 0.01$). Further source analysis indicated BBOEP and DPHP mainly derived from biodegradation of their parent compounds. As the most recalcitrant OPFRs in WWTPs, the relatively high concentrations and detection frequency of BCEP, BCIPP and BDCIPP suggested that they mainly originated from outside sources of WWTPs. The estimated total emission fluxes of OPFRs and emerging metabolites via land-application sludge in China were 1320 and 536 kg y^{-1}, respectively. Further risk assessment by risk quotient (RQ) values in sludge-applied soils indicated that low or medium risks for most OPFRs and OPFR metabolites except tris(methylphenyl) phosphate (TMPP). The nationwide accumulations of PFRs and widespread occurrences of emerging metabolites in sludge raise environmental concerns about these contaminants.

124 Fate of organophosphate esters in polar bears and ringed seal prey using in vitro metabolism and structure-activity relationships

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The Arctic is a sink for anthropogenic substances vialong-range transport. This includes organophosphate esters (OPEs) which have been quantified in Arctic atmosphere. OPEs are additives in consumer products functioning as flame retardants replacing polybrominated diphenyl ethers (PBDEs). Exposure to Arctic wildlife is of concern and several OPEs have been detected in Arctic biota at low concentrations. This suggests rapid metabolism of OPEs from high environmental exposure. The present study provides metabolism and residue data of a subset of OPEs in polar bears (*Ursus maritimus*) and ringed seals (*Pusa hispida*) collected from the East Greenland area of Scoresby Sound. *In vitro* metabolism data strongly suggests that the parent organophosphate (OP) triester metabolic rates are more rapid in East Greenland polar bears than ringed seals. For example, TPHP depletion was determined to be statistically significant after 40 minutes ($p< 0.015$) using polar bear microsomes while was nonsignificant over the 100-minute assay using ringed seal microsomes. Thus, it appears that polar bear exposure to OPEs is likely to be a function of both dietary and environmental sources. Additionally, the difference in rate of OP triester depletion can be used to better describe the role of structure on the fate of OPEs. These findings also show that the metabolism capacity of ringed seals and polar bears effects corresponding residue levels. Furthermore, the present research provides toxicokinetic data on priority and environmentally relevant OPEs identified by the Canadian Chemical Management Plan (CMP) in this top predator-prey interaction.

125 Tetrabromobisphenol-A-bis(dibromopropyl ether) Flame Retardant in Herring Gull eggs from the Laurentian Great Lakes: Temporal and Spatial Patterns

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Herring gulls (*Larus argentatus*) are opportunistic scavengers with a varying terrestrial and aquatic diet (i.e. fish, mammals, songbirds, and garbage) and are exposed to chemical pollutants through their food. This variable diet makes them useful for identifying possible routes of exposure to persistent organic pollutants (POPs). Eggs from fifteen colonies on the Laurentian Great Lakes have been collected annually since the 1970s and are used to report on various legacy POPs and increasingly for the identification and trends determinations of new and emerging contaminants, e.g. flame retardants (FRs). With recent regulations on production and use of various FRs, including polybrominated diphenyl ethers and hexabromocyclododecane, it is important to monitor for the presence of potential replacement FRs in biota. Tetrabromobisphenol-A (TBBPA) is among the most highly produced FRs, and increasingly more TBBPA derivatives are being produced including TBPPA–bis(dibromopropyl ether) (TBBPA-BDBPE). Limited information exists on TBBPA-BDBPE in environmental biota. The present study focuses on spatial and temporal patterns of the TBBPA-BDBPE in herring gull egg pools and individuals collected from colonies on the Laurentian Great Lakes. TBBPA-BDBPE was quantifiable in egg pools from all fifteen colonies sampled in 2017 and retrospective analysis of archived eggs (2000-2017) indicated that TBBPA-BDBPE concentrations were greater in eggs collected in more recent years. Data for TBBPA-BDBPE were also generated for individual herring gull eggs and dietary markers, i.e. fatty acids and stable isotopes, in eggs revealed likely exposure pathways of this alternative FR.

126 Effect of Removal of Flame-retarded Nap Mats on Indoor Flame Retardant Levels in Childcare Environments

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Nap mats are a significant item in childcare settings used for the children's naptime. Typically, they are one- to two-inch thick mattresses made of polyurethane foam encased in a vinyl cover. Childcares that serve young children in full-day programs generally use, and store, one nap mat for each child. For this study, we conducted an intervention, replacing flame-retarded nap mats with flame-retardant-free mats, and measured concentrations of flame retardants in indoor air and dust before and after replacement. Seven childcare centers in Seattle, USA, were recruited to participate in the study using the following criteria: regular use of nap mats, serving low-income families, and minimal presence of other foam-containing items. Foam from existing in-use nap mats from each center was analysed to confirm the presence of flame retardants. Foam and fabric from replacement mats was also analysed for flame retardants to identify flame-retardant-free products. Existing mats were replaced with flame-retardant-free mats in six centers. Nap mat foam from four childcare centers contained components of a FireMaster® mixture, 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EHTBB) and bis(2-ethylhexyl) tetrabromophthalate (BEHTBP) at percent levels by weight. Smaller quantities of organophosphate esters (OPEs) consistent with a FireMaster® mixture were also present, such as triphenyl phosphate (TPhP), tri-m-tolyl phosphate (TMTP), and tris(4-butylphenyl) phosphate (TBPP). The nap mat from childcare center 3 (CC3) was primarily flame retarded with TDCIPP, but also contained smaller concentrations of a FireMaster-like mixture. Nap mats from CC1 were flame retardant free. The sum of flame retardant concentrations found in nap mat foams ranged from 5.4 – 8.4

% by weight. Concentrations of EHTBB in dust were 1300 - 6800 ng/g lower at five centers after replacing nap mats, four of which contained EHTBB. BEHTBP concentrations were lower in post-replacement dust at all six centers, with levels decreasing by 1.3 - 91 ng/g. Concentrations of TDCIPP decreased by 2100 ng/g in dust collected post-replacement at CC3. Differences in the concentrations of both EHTBB and TBPP in the dust were statistically significant ($p< 0.05$) and BEHTBP statistically significant at a lower confidence limit ($p< 0.1$), suggesting that the nap mats were the source of the FireMaster® in dust. By replacing the nap mats, the FireMaster® source was removed and concentrations were significantly lowered.

127 Activation of PAPRg and Disruption of Progesterone Synthesis of EHDPP in Human Placental Choriocarcinoma Cells: Comparison with TPhP

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2-Ethylhexyl diphenyl phosphate (EHDPP), an organophosphate flame retardants (OPFRs), is frequently detected in human blood. In this study, sensitive dual-luciferase reporter gene assay and molecular docking were used to investigate the activation of EHDPP to human peroxisome proliferator-activated receptor gamma (PPARG). Results show that EHDPP exhibited stronger PPARG activation (EC_{20}: 2.04 μM) than triphenyl phosphate (TPhP) (EC_{20}: 2.78 μM). EHDPP upregulated the gene expression of 3β-hydroxysteroid dehydrogenase type 1 (*3β-HSD1*) in human placental choriocarcinoma cells in a dose-dependent manner, and the lowest observable effective concentration was 10 μM, lower than that of TPhP (20 μM). EHDPP significantly altered progesterone secretion at a lower concentration (10 μM) than that of TPhP (20 μM), and both EHDPP and TPhP significantly promoted human chorionic gonadotropin (hCG) production at 20 μM. Furthermore, inactivation of PPARG by either pharmacological inhibitor (GW9662) or small interfering RNA (siRNA) abolished the change in progesterone secretion and gene expression in the cells exposed to EHDPP, suggesting that the PPARG signaling pathway plays a role in the upregulation of progesterone by the two OPFRs. This is the first report to show that OPFRs can alter the biosynthesis of progesterone in the placenta, which could affect female reproduction and fetal development.

128 Impact of Brominated Flame Retardant Chemical Structure on Binding Affinities for Human and Zebrafish Thyroid Receptor Beta

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Thyroid hormones are essential for normal fetal and juvenile development and normal adult physiology in all vertebrates. The biological actions of THs are mediated though the binding and activation of thyroid hormone receptors (TRs), which control the expression of target genes. Brominated flame retardants (BFRs) are implicated as thyroid disrupting chemicals in humans and wildlife due to their ability to mimic THs. However, the influence of chemical structure on TR binding is not well understood. In this study, a competitive binding assay was established using recombinant zebrafish and human thyroid receptor beta (TRb) to examine the role of chemical structure on TRb affinities for three major BFRs and their structural analogues: BDE 47, tetrabromobisphenol A (TBBPA), and 2,4,6-tribromophenol (TBP). Structure variations included presence/absence of halogens, species and degree of halogenation, number of aromatic rings, presence/absence of hydroxyl groups, and aromatic ring linkages. Nearly all tested compounds exhibited some affinity for both receptors, and the affinities between species were highly correlated. Affinity increased with halogen size and degree of halogenation, with tetraiodobisphenol A exhibiting the highest affinity for both receptors. Similar to other studies, a hydroxyl group is required for binding, as BDE-47 displayed no binding; however, the hydroxylated PBDEs bound both receptors with sub-micromolar affinity. Contrary to previous studies, these results indicate that hydroxyl group position on the ring had little influence on binding affinity. The TBP variants had lower affinities compared to the two ring structures, however this could be compensated by substituting larger halogens and increasing the degree of halogenation on the ring. Linkage groups between the aromatic rings influence TRb affinities, as replacing the propyl linkage of TBBPA with the sulfone of TBBPS significantly decreased TRb affinities. In general, binding affinities were highly correlated with molecular weight, volume, and hydrophobicity of the compounds. Compounds with the lowest affinities, such as bisphenol A and bisphenol S, had few or no ring substituents beyond hydroxyl groups. Overall, this study identified structural features of BFRs that impact TRb binding affinities and are conserved between species. Further studies exploring how the identified structural modifications impact downstream receptor signaling and adverse outcomes are warranted.

Soil Contaminants – Fate, Bioavailability, Environmental Toxicology – Part 1: Applications in Ecological Risk Assessment

129 Using Diffusive Gradient Thin Films (DGT) and Ion Exchange Technique (IET) to Relate Bioaccessibility and Phytotoxicity of Rare Earth Elements

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The extractive mining and use of rare earth elements (REEs) has increased in recent decades, thus increasing deposition of emissions and waste associated with the mining and manufacturing processes to surrounding environments. There are insufficient data describing the ecotoxicity of this group of metallic elements to establish regulatory soil quality guidelines. Unlike data rich metals such as Cu and Ni, toxicity associated with REEs has not yet been well demonstrated to relate to the soil REEs that are readily available for uptake from the soil (also known as bioaccessibility). Bioaccessibility of REEs will be measured using Diffusive Gradient Thin Films (DGT) (Figure 1) and Ion Exchange Technique (IET) to determine which is more predictive of the true exposure of plants, thus better correlated with toxicity. It will also be compared to the theoretical values predicted by the modeling program WHAM 7 with the expectation is that the values determined by IET and WHAM will be more similar than DGT and WHAM. Doing so will fill in data gaps that will contribute to the development of federal soil quality guidelines as well as site specific risk assessment protocols. Results of ongoing research will be presented.

130 Phytotoxicity of bismuth on germination and growth of perennial ryegrass (Lollium perenne)

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Phytotoxicity of Bi citrate and Bi nitrate on perennial ryegrass (*Lollium perenne*) were evaluated using filter paper and soil tests. Endpoints include seeds germination, root and shoot growth. The results showed that Bi nitrate seemed to be more toxic than Bi citrate, and root growth more sensitive than seeds germination and shoot growth. Filter paper test indicated that Bi nitrate decreased significantly root elongation at tested concentration ≥ 30.4 mg/L and seedling germination at 485 mg/L. Whereas, Bi citrate decreased significantly root elongation and seedling germination at concentration ≥ 99.52 mg/L and at 398.08 mg/L, respectively. Data from artificial soil spiked with Bi nitrate indicated a significant reduce on root wet mass at 485 mg/kg soil and not significant effect was observed on shoot wet mass and germination at concentration ≤ 485 mg/kg soil. In natural sandy soil, Bi nitrate reduced significantly root wet mass and root elongation at concentration ≥ 4.8 and 48.5 mg/kg soil, respectively, while no effect were observed on seeds germination and shoot mass. Sandy soil spiked with Bi citrate showed no significant decreased in root growth, root growth and germination. The Bi availability varied with the physicochemical soil characteristics and the toxicity of Bi nitrate/citrate on perennial ryegrass varied with the matrix in the following order: filter paper > natural sandy soil growth > artificial soil.

131 Influence of Climatic Conditions on subsurface pesticide fate and transport

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The intensive use of pesticides has substantially improved the agricultural production, thus providing improved quality and increased quantity of food for the growing population. The widespread contamination of pesticides in the groundwater and the associated toxic effects has become a serious environmental problem. The present study focuses on climate drivers such as precipitation and soil organic carbon changes on subsurface pesticide transport. Sorption, transformation and degradation studies were carried out in batch and column studies for agricultural soil collected from field site in India and USA. A model is developed considering Richard's equation for unsaturated zone and advection-dispersion-sorption-biodegradation equations. Initially the model was validated with the published data from literature and sensitivity analysis was carried out to understand the different factors affecting transport. Further, the columns experiments are used to calibrate and validate the model. This study makes an attempt to address the problems associated with the transport of pesticides leading to groundwater contamination, taking into consideration the changing climatic and agricultural conditions. The work helps in better understanding the change in mobility and behavior of pesticides during its transport in subsurface system for better assessment of risks and undertaking efficient remediation measures by considering the inclusive effects of precipitation and soil organic carbon changes.

132 Partitioning Model of Explosives as an Environmental Impact Assessment Regulation Tool in Colombia

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The Environmental Impact Assessment is a regulation tool to prevent the effects generated by an activity and control to an impact generated by a situation that involves remediation actions. In Colombia, the peace agreement between the government and groups outside the law, have permitted that in large zones in the country begin the demine process to restore the land to the farmers that came out many years ago by the violence. In addition to that, few months ago the first environmental soil regulation was enacted by the environmental authorities and it must be fulfilled. Due this, is necessary a regulation tool to determine the ecotoxicological impact that explosives and heavy metals as constituting materials of the mines have caused in the soils, but is well known that this type of studies is very costly due to the variability of soil characteristics and Colombia does not have the necessary infrastructure to develop them. For these reasons, this research focused on use the Multilineal Model, which was developed for twenty-five soils with a wide range of physicochemical properties (Total Organic Carbon COT 0.07-18.2%, pH 3.8-6.9, Clay 4-38.6 %) to predict the environmental fate of explosives in soils by calculating the partition coefficient of them to determine the fate of some explosives in two demined towns in Colombia and thus generate remediation proposals according to the concentrations of these chemicals. The use of the model successfully predicted its environmental fate, improving its accuracy by a factor of 2, compared to the traditional normalization model. Methodology involved surveys to certified deminers by the Colombian National Army, to determine the most common explosives and materials used in the mines and later this information was used in an evaluation matrix to determine the environmental impact under controlled conditions. The data showed the soil impact in those towns. Is the first time in Colombia at the soil level was a similar process, which showed that it can provide elements of control in public health and safety in a relatively simple and very precise way.

133 Bioaccessibility, bioavailability and toxicity of bismuth on the earthworm Eisenia andrei

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Bismuth (Bi) is used increasingly to replace lead in several ind*u*strial applications including the production of alloys and munitions formulations. However, little information is available on the environmental fate and ecological effects of Bi. This paper summarizes the acute toxicity (LC50=416 mg Bi/kg dry soil) and biaccessibility of Bi, and describes bioavailability and chronic effects of bismuth on the earthworm *Eisenia andrei*. The bioaccessible fraction of Bi in soil was determined using KNO_3 soil extraction. In reproduction tests, adult earthworms were exposed to natural sandy soil spiked with Bi citrate. The tested soil concentrations were based on the previous acute toxicity study and the measured concentration of Bi ranged from average 75 to 289 mg/kg. Results indicate that Bi significantly decreased reproduction parameters at concentrations ≥ 116 mg/kg. The number of hatched cocoons and juveniles were sensitive endpoints. Bismuth did not affect *E. andrei* growth and survival at the tested concentration, and had little effect on phagocytic efficiency of coelomocytes. The low immunotoxicity effect might be explained by other mechanisms *i.e.* Bi sequestered by metal-binding compounds as metallothioneins. Indeed, after 28 days of exposure Bi concentrations in earthworm tissue increased with increasing Bi concentrations in soil, and reaching a stationary state at 21.37 mg Bi/kg dry tissue for 243 mg Bi/kg dry soil. Data also indicate that after 56 days of exposure, the average fractions of Bi available in soil without earthworms varies from 0.0051 to 0.0229 mg/kg, while in presence of earthworms. Bi concentration ranged between 0.310 to 1.347 mg/kg dry soil. This increase in metal bioaccesssibility could be explained by the mucus and chelating agents produced by earthworms and the microorganisms in the soil or/and earthworm gut as well as the dermal and ingestion routes of uptake.

134 Is habitat quality a better predictor of mite survival and reproduction than metal bioavailability in different contaminated soils?

K. Jegede, University of Saskatchewan / Toxicology Centre; B.A. Hale, University of Guelph / School of Environmental Sciences; S. Siciliano, University of Saskatchewan / Soil Science

The responses of organisms in soil ecotoxicity tests are often determined by the bioavailable or bioaccessible concentrations of contaminants they are exposed to. However, the direct effect of habitat quality on the performance or response of organisms in different contaminated soils is often neglected. Habitat quality is a measure of extent to which habitat promotes individual and population fitness. This study assessed the effect of habitat quality on mite, *Oppia nitens* exposed to different contaminated soils which was corrected for bioavailable metals. Forty-seven (47) soils were ranked into habitat quality by summing up the scores of enchytraeid and collembola survival and reproduction with the plant biomass in each of the soils. From the 47 soils, nine (n=9) soils were selected in groups of three based on high, medium and low habitat quality. The nine soils were dosed with low to high concentrations of zinc and the mites were exposed to the soils for 28 days. Mite survival and reproduction were assessed after 28 days and bioavailable metal concentrations in the soils were determined. Using GLM, the habitat quality will be compared with metal bioavailability to see which predicts mite reproduction better. The significant differences between the bioavailable corrected zinc EC50s will also be assessed between the three habitat qualities.

135 Soil bacterial responses to metal mixtures: The role of metal species

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The fixed ratio ray design is an economic way of carrying out metal mixture experiments because it allows the estimation of both additivity and interactions. In using this design, metal concentrations should be fixed in specific ratios. In soil laboratory experiments, metal mixture studies are usually carried out with metals dosed as salts, followed by leaching with artificial rainwater to remove salts. In the leaching process, metals are lost unevenly, which contradicts with the cardinal phenomenon behind the fixed ratio ray design. To keep fixed metal concentrations and to avoid leaching, a salt control is usually added to the experimental design. Toxicity modifications from the metal salt mixture are difficult to be accounted for by the salt control. Hence, an alternative method of dosing that allows fixed ratio tests had to be determined. Two proposed alternatives were metal oxides and annealed oxides (metal iron oxides), which are abundantly found in both aged metal salt spiked soils and field metal contaminated soils. The toxicity of the oxides to soil microbes and enzymes were tested and compared to the salts. The experiment was conducted with three Canadian soils, three metal species, five fixed metal mixture rays, and five metals at one dose level. The activity of the soil enzymes ammonia monooxygenase, glucosidase, phosphatase and sulphatase were measured. Results showed that, soil enzymes are more sensitive to metal salts, followed by metal oxides and then annealed oxides. Sulphatase was however more sensitive to the annealed oxides compared to the metal oxides in one of the soils.

136 Impacts of silver-grapheneoxide on soil microbial activity and composition

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Silver-graphene oxide (Ag-GO) is a nanocomposite applied in various fields including environmental engineering, bioengineering, and electrochemistry due to its unique material properties. As the use of Ag-GO is increasing, it may enter soil ecosystems, and thus it is important to understand its potential impacts on the soil environment. We investigated the response of microbial communities in soils exposed to Ag-GO that was synthesized by glucose reduction. Soil enzyme activities, microbial biomass, and inorganic N concentrations were analyzed and pyrosequencing was conducted to determine the changes in the activity and composition of soil microbial communities. In soils treated with 0.1 - 1 mg Ag-GO g^{-1} soil, the activities of C-cycling enzymes, β-glucosidase, cellobiohydrolase, and xylosidase, decreased up to 80%. In addition, soil NO_3^- concentration decreased up to 82%, which indicates that nitrification was inhibited. The relative abundance of *Acidobacteria* and *Cyanobacteria* in soils exposed to Ag-GO was lower than that in control soil. On the other hand, the relative abundance of *AD3* and *Firmicutes* tended to increase in soils treated with Ag-GO. These changes in bacterial community composition are in accordance with decreased microbial activities associated with C and N cycling. Meanwhile, microbial biomass showed no distinct change under Ag-GO treatment. Our study indicates that Ag-GO can negatively affect microbial communities in soils, and these results can be useful in establishing regulatory guidelines for the release of nanocomposites such as Ag-GO to soils.

Fate and Effects of Metals – Biogeochemical Perspective

138 Abiotic mechanisms of mercury (II) chloride reduction on surfaces

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Mercury (Hg) is a ubiquitous, toxic and bioaccumulative environmental contaminant that undergoes reaction to change speciation and environmental fate. Some areas, like the Oakridge National Laboratory Y-12 National Security Complex (TN, USA), have experienced historical contamination of surrounding soils with Hg, but remediation of such sites is hindered by poor mechanistic understanding of Hg reactions in soil. Specifically, non-volatile Hg species in soil can undergo reduction to form Hg(0), which can then be lost to the atmosphere; however, the mechanism of this reaction is not known. We have used a combination of computational modeling and laboratory experiments to identify reduction mechanisms for one environmentally relevant Hg species, mercury (II) chloride ($HgCl_2$), in soil-like environments. Computational modelling using Gaussian software suggests that $HgCl_2$ reduction might include a unimolecular dissociation driven by scissoring of the chlorine bonds to form Hg(0). Based on the energy of excitation suggested in these computational results, we hypothesized that ultraviolet-B (UVB; 280 – 320 nm) radiation would provide the energy necessary to cause dissociation of $HgCl_2$ to Hg(0), in the absence of a secondary electron donor. To test this, clean silica sand was spiked with an aqueous solution of $HgCl_2$, and exposed to full spectrum radiation, while the flux of Hg(0) from this sand was quantified over time. Filters were applied to remove ultraviolet radiation, and preliminary results support the molecular modeling data, suggesting that UVB radiation is particularly important in the reduction of $HgCl_2$ in sand; however, in the absence of UVB, and indeed even when both UVB and UVA (320 – 400 nm) radiation are filtered out, we have still observed some reduction of $HgCl_2$ in sand. Experiments are underway to determine if this unexpected behaviour can be observed in natural soil, and/or soil which has been spiked with $HgCl_2$, or if this is specific to the sand matrix.

139 Arsenic concentration variability in newly constructed drinking water wells in Minnesota, USA

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Arsenic is a naturally-occurring contaminant adversely affecting drinking water quality sourced from groundwater in geologically diverse aquifers in Asia, Europe, Africa, and North and South America. Minnesota has an estimated 250,000 domestic water well users with drinking water arsenic concentrations above 10 µ/L (elevated arsenic). The State of Minnesota revised the well code in 2008 to include testing all new potable wells for total arsenic (in addition to nitrate and bacteria), but the code does not require any particular sample collection method or sampling point. To better understand the influence that sample collection methods, sampling point, and timing have on measured arsenic concentration, arsenic concentrations were measured in 250 newly constructed wells over one year in several counties known to have prevalent elevated arsenic concentrations in groundwater. Study samples were collected in the following ways: 1) total arsenic samples (unfiltered) collected by well drillers in each respective driller's common practice (from the drill rig or from plumbing); 2) initial total and dissolved (filtered) arsenic samples by MDH staff replicating driller sample timing and sampling point; 3) total and dissolved arsenic samples by MDH staff 3-6 months after well construction; and 4) total and dissolved arsenic samples by MDH staff 12 months after well construction. Field parameters and redox-sensitive constituents (arsenate, arsenite, nitrate, manganese, iron, and sulfate) were also collected during each round. Most arsenic is present at As(V). Arsenic, iron, and sulfate concentrations combined with pe indicate that three primary arsenic mobilization mechanisms are active: a) reductive dissolution of iron, b) rapid oxidation of iron sulfides and re-precipitation of Fe as (oxyhydr) oxides, and c) incongruent dissolution of iron sulfides (rapid oxidation and re-precipitation of iron as oxides). Initial total arsenic concentrations varied significantly from later sample concentrations. In contrast, initial dissolved sample concentration varied much less over time. Over one year, total initial arsenic samples switched between categories of above or below the 10 µg/L drinking water standard in more than 13% of wells. Dissolved arsenic samples switched between categories in only 7% of wells. Filtering initial arsenic samples reduces concentration variability over time and improves the reliability of the public health message.

140 Colloidal Transport of Metals in the Subterranean Estuary

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In surface estuaries, the transport of organic matter, iron, and trace metals to the sea is modified by the flocculation and removal of colloids, functionally defined as particles from 1 nm to 1 µm in diameter. Like surface estuaries, subterranean estuaries (STE; the subsurface mixing zone of outflowing fresh groundwater and infiltrating seawater) exert considerable control on the composition of water reaching the coastal ocean. However, little is known about the importance of the colloidal phase for trace metal transport in these systems. We investigated how surface charge and composition of colloidal particles in an STE changed along the salinity and redox gradients, and how these properties affected the transport of the redox sensitive metals (RSMs) Mo, U, V, and Cr. Colloidal surface charges (as determined by electrophoretic mobility) were similar to those found in surface estuaries, ranging between -0.1 and -2.2×10^{-8} $m^2\ V^{-1}\ s^{-1}$. The abundance of colloidal particles, as determined by the concentrations of DOC, Fe, and Mn in the truly dissolved (< 3 kD), small colloidal (3-30 kD), and large colloidal (30 kD to 0.45 µm) size fractions, generally followed the distribution and salinity mixing behavior of the total dissolved concentrations. In contrast to surface estuaries, the proportion of colloidal DOC, Fe, and Mn were directly correlated to salinity. The particular geochemistry of each RSM determined its partitioning into the colloidal pools. The fraction of Mo, U, and V in the small colloidal phase was inversely related to salinity (similar to surface estuaries), but PCA of environmental variables (salinity, Eh, electrophoretic mobility) and colloidal composition (DOC, Fe, and Mn) suggested that different controls were important for the partitioning of each RSM into distinct colloidal size fractions. This work showed that up to 70% of trace metals are associated with the colloidal phase in the STE, and further suggests that other particle reactive elements may have similar enhanced mobility in the STE.

141 Developing biotic ligand model for predicting toxicity of copper and cadmium to Daphnia magna: A new method with less work but better performance

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The biotic ligand model (BLM) improves our understanding of the effects of water chemistry on metal toxicity to aquatic organisms. However, BLM is currently developed for only a few metals in several model organisms. The scarcity of available BLM hinders the progress of using this tool in developing site-specific water quality criteria by environmental regulatory authorities. One of the reasons for the scarcity of BLM is probably the huge amount of experiments required for developing BLM. For example, 38 toxicity tests and 6660 individuals of *Daphnia magna* were used for developing a BLM of Cu according to the method of De Schamphelaere et al. (2002). Developing a BLM is essentially measuring a set of stability constants (K) for the binding of the toxic metal ion and competition cations to the biotic ligand. In this study, we developed a new approach for measuring the K values for predicting the toxicity of copper (Cu) and cadmium (Cd) to *D. magna* in the framework of the toxicokinetic-toxicodynamic (TK-TD) model. Specifically, a toxicity test of Cu or Cd was firstly conducted in water with moderately low concentrations of major cations. Then, a constant Cu or Cd concentration (close to the 50% effective concentration) was used in the following tests with varying concentrations of major cations (i.e., Ca^{2+}, Mg^{2+}, Na^+, K^+, and H^+) to quantify their competition effects. The survivorship of daphnids were monitored every 6 to 8 h during the 48-h exposures. Elevated survivorships were observed with the increasing concentration of competing cations. Our assumption for the observed protective effects was that the competing cations affected the binding of Cu^{2+} (or Cd^{2+}) to biotic ligands and subsequently lowered the uptake rate constants (k_u) of the metals. The values of k_u were estimated using the TK-TD model. The K values were estimated through regression analysis between k_u and concentrations of competing cations. Using the new approach, the measured K values were similar to those reported in the literature, although the number of experiments and test organisms needed were ~15% of the traditional approach. Moreover, the BLMs we developed are kinetic, i.e., time is included as a variable; therefore, the model parameters are not constrained to the duration of toxicity tests from which they were calibrated and can be used under exposures of fluctuating metal concentrations.

142 Dissolved nickel partitioning to five geochemically distinct oxic sediments

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Nickel bioavailability in sediment is modified by naturally occurring ligands that can complex Ni and reduce its toxicity. Many of the environmental quality standards for Ni in sediment consider reduced sulfur and organic matter as important modifying factors, but compounding evidence suggests metal oxide minerals can alter sediment Ni bioavailability. We used controlled laboratory assays with five geochemically diverse sediments to study the binding of Ni to oxidized sediment ligands. We incubated field-collected surface sediments and buffered reconstituted freshwater solution with dissolved Ni (0.5, 2, or 5 mg L^{-1}) under different pH conditions (pH 5, 7, or 9) for 28 days in a fully factorial experimental design (modeled after OECD Test 308). Overlying water Ni concentrations declined rapidly in most treatments, and reached an equilibrium concentration within 1.5 days. Slower Ni removal occurred when overlying water was pH 5 and when Ni loading was low (0.5 mg L^{-1}). At equilibrium, sediments sorbed 44–100% of the added Ni, and approximately two thirds of the test conditions were predicted to remove >70% of the added Ni. Ni dynamics in the overlying water were strongly correlated to the dynamics of Ni sorbed to metal oxide minerals. Increased Ni removal (i.e., greater proportion of added Ni sorbed to sediment) was correlated to the affinity of Fe for Ni measured as both the Ni:Fe ratio in hydrous ferric oxide (HFO)-specific extractions and the Ni-HFO partitioning coefficient ($K_{Ni\text{-}HFO}$). Ni removal efficiency was negatively correlated with acid-volatile sulfide (AVS) and organic matter in sediment, which supports the hypothesis that metal oxides are the primary ligands for Ni in surface sediments. Although the specific affinity of metal oxides for Ni was strongly correlated with overlying water Ni removal, bulk mineralogical sediment characteristics (e.g., total Fe, total HFO) were either uncorrelated or negatively correlated with Ni removal. Particle size was positively correlated to Ni removal, which suggests that metal oxides on the surfaces of larger particles have greater affinity for Ni. These data show that Ni removal from overlying water by surface sediments is rapid and complete in non-acidic (i.e., pH > 6) conditions, and the capacity for Ni removal by sediment is related to properties of metal oxide minerals.

143 Metal partitioning to oxic natural sediments and removal from water

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Metals usually enter aquatic ecosystems in an oxic environment and associated with particles. It is important to understand this initial fate process in regards to partitioning, speciation and resulting biological effects. In addition, current European Union regulations mandate a hazard evaluation, which includes the assessment of Rapid Degradation (greater than 70% within 28 days), which for metals equates to metal removal from the water column. The Transformation/Dissolution Protocol (OECD 29) is an established method that can be modified to examine metal removal from the water column. We conducted a series of laboratory evaluations to address the following questions: Are copper (Cu) and nickel (Ni) removed from the water column of freshwater systems and if so, what is the rate of removal? How do various test method conditions affect metal removal, using OECD method 29? What sediment characteristics affect metal removal and which show a reasonable worst case (RWC) condition? What is the mechanism for metal removal, and are metals released into overlying waters upon subsequent resuspension? Method parameters evaluated included: sediment type and loading rate, pH control, metal loading rate, pre-incubation of sediment, and resuspension. Chemical analyses included dissolved Cu, Ni, and Fe, dissolved oxygen (DO), pH and AVS-SEM of sediments. Multiple dried vs. non-dried sediments were tested in batch reactors for both 96 h and 28 d tests. Dry Buffalo River sediment typically removed 70% Cu and Ni from the water column at 1 mg/L loading. No significant differences were noted between incubated and non-incubated sediments. Higher sediment loading rates removed metals faster as expected. Sediment type and loading rates affected pH, which started at 6.0. Cu removal (96 h) and resuspension (1 h post 96 h) resulted in no significant increase in Cu, but did elevate Fe concentrations. The results show that 70% removal of Ni and Cu from the water column is feasible with a variety of sediments and variable conditions.

144 Determination of the concentration of free Ni ion ([Ni2+]) in seawater to explain Ni toxicity

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Nickel (Ni) toxicity in seawater is an increasing concern because of coastal Ni mining and processing activities. Determining Ni speciation is vital to the understanding of Ni toxicity. The free ion (Ni^{2+}) is presumed to predict toxicity but experimental evidence to evaluate this assumption in sea water is lacking. Our study aims to analytically determine [Ni^{2+}] and compare to measured toxic effects concentrations. Defined solutions of artificial seawater (ASW) containing different model ligands (i.e. citric acid, EDTA, L-Tryptophan, glutamic acid, and histidine) were titrated with Ni to determine speciation. In addition, Ni speciation was determined in real saltwater samples of diverse geographic origin. The divalent Ni-free ion in these synthetic or real seawater samples was quantified using Ion Exchange Technique (IET) with Ni measured by Graphite Furnace Atomic Absorption (GFAA). To our knowledge, this is the first time the IET has been used for Ni in seawater. The equilibrium time, calibration coefficient, and the amount of acid for elution were optimized for the IET experimental specifications in seawater. The measured [Ni^{2+}] values were compared with model predictions (i.e. Visual Minteq) for evaluating the feasibility and applicability of the IET method for Ni in seawater. For the most part, the IET-measured [Ni^{2+}] agreed very closely with model predictions. In the same defined solutions, 96-hour Ni embryo toxicity tests were performed for a sea urchin (i.e. *S. purpuratus*). The dose response curves were expressed both as total dissolved Ni concentration ([$Ni]_T$) and free Ni concentrations from IET ([Ni^{2+}]). If the Ni toxicity is explained by [Ni^{2+}], all the toxicity response curves of different model ligands will overlap and this was in fact observed for most of the samples. Our results show that (1) IET-GFAA is a suitable technique for determining [Ni^{2+}] in sea water and (2) Ni toxicity is explained by Ni^{2+}. The results are significant for the development of marine biotic ligand model (BLM) for Ni toxicity assessment.

Immunotoxicology – Impacts of Contaminants on Immune Function and Susceptibility to Disease

145 Nanoparticle protein corona formation in human blood and effects on macrophage phagocytosis

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When nanoparticles (NPs) breach protective external barriers and infiltrate biological compartments such as blood vessels, they quickly acquire a diverse mixture of blood proteins and other biomolecules that bind to the NP to form the biomolecular protein corona. Nano properties such as charge, surface area and size are key characteristics that likely dictate the profile of proteins that are present, however, it is the protein corona itself that establishes the “biological identity” of the NP and encodes important information about the interface that forms between the NP and a cell surface. Biological responses to NPs would therefore be a function of this interface and could be traced to the corona profile. However, in most in vitro nanotox studies, the corona is not evaluated when interpreting toxicological outcomes, but focus instead on NP properties that were characterized post-synthesis, and without a protein corona. Therefore, it would be more precise to evaluate the biological/toxicological outcomes of NP exposures from the bio-corona perspective, rather than from its original synthesized form. Recent studies have shown that the same NP can induce different biological outcomes, depending on the presence or composition of the protein corona, and others have shown that protein coronas are composed of immune-activating proteins. Therefore, understanding the composition of the corona is key to understanding the effects NPs impart on immune cells. Despite studies showing that immune cells are taken up by protein-coated NPs, what has not been examined are the effects of protein coronas on the opsonisation and phagocytosis of pathogens by immune cells. Therefore, we used phagocytic immune cells to compare the effects that different metal-oxide NPs have on the opsonisation and phagocytosis of pathogens. We also profiled human serum proteins that form the NP-protein corona to correlate with potential biological effects. Western blot results showed there were significant variations in the protein corona profiles of our NPs, which contained several immune proteins, including IgG, transferrin, complement and fibrinogen, plus clotting proteins. The NPs also inhibited normal blood clotting, relative to controls. Macrophage phagocytosis results showed that pre-coating NPs with serum proteins potentiated cell uptake of GFP-*E. coli*, indicating that the presence of NP in blood may be activating immune functions via the protein corona, and disrupting clotting.

146 Determination of suitable host-pathogen combinations to detect the chemical effects on susceptibility to infectious disease

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In classical methods for evaluating the immunotoxicity of a chemical in fish, the activities of leukocytes, isolated from chemically exposed fish or exposed to the test chemical in vitro, were analyzed without immune stimulation. Even though these methods can detect the effects of chemicals on leukocytes, neither method provides direct evidence as to whether the observed effects alter the susceptibility of the fish to pathogen infection. Therefore, researchers have begun to analyze the responses to pathogen infection of fish exposed to test chemicals. We also tested immunomodulatory effects of some pollutants using several different host-pathogen combinations, Japanese flounder (Paralichthys olivaceus) and viral hemorrhagic septicemia virus (VHSV), Japanese medaka (Oryzias latipes) and betanodavirus, or common carp (Cyprinus carpio) and bacteria of genus Aeromonas. Each test method showed that pathogen infection-associated mortalities were significantly increased by exposure to the test chemicals. The above mentioned three fish species are easy to maintain, however, each method has its own disadvantages. Since Japanese flounder is a marine fish and VHSV can cause mortality in fish at relatively low temperature (< 15° C), special facilities are required to use the host-pathogen combination. As for medaka and betanodavirus, the virus can induce mortality only at fry stage, therefore, it is difficult to analyze the immunological parameters in such small size fish. Finally, we decided to use the combination of carp and Aeromonas salmonicida. We selected carp as the host fish because it is easy to maintain in a laboratory, the carp immune system is relatively well studied, and several different pathogens have been isolated from carp. Additionally, collecting head kidney and enough blood for measurement of immune system parameters is easy. We chose A. salmonicida as the pathogen because it is easy to culture and it can induce mortality in host fish by bath infection. The results of experimental infection of carp with A. salmonicida are highly reproducible across laboratories. Furthermore, A. salmonicida disease is not a specified disease by World Organisation for Animal Health (OIE). The factors discussed above make the combination of carp and A. salmonicida suitable for the analysis of the effects of chemicals on host-pathogen relationships. To increase the throughput of the test, we are now optimizing the method for small size carp (body weight < 2.0 g), and the data will be presented.

147 An Overview of Oil Immunotoxicity in Three Commercially and Recreationally Important Fish Species

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The 2010 Deepwater Horizon oil spill resulted in nearly 5 million barrels of crude oil being released into the Gulf of Mexico ecosystem, potentially impacting many commercially and recreationally important fish species. While a variety of research has been undertaken to explain the impacts of this spill, the study of oil-induced impacts on immunocompetence in fish remains lacking. The alteration of immune parameters can lead to serious adverse consequences, such as increased susceptibility to bacterial infections. To examine how exposure to slick A Macondo oil impacts the immune systems of fish species, red snapper (*Lutjanus campechanus*), Atlantic croaker (*Micropogonias undulatus*), and red drum (*Sciaenops ocellatus*), juvenile fish were exposed to HEWAF over a 9-18 day period, then challenged with exposure to the known fish pathogen *Vibrio anguillarum*. For all three species, there were no significant differences in mortality amongst treatment groups, however, significant differences in length and weight were seen in red drum, with the groups exposed to the highest oil concentrations (both with or without pathogen exposure) differing significantly from the other groups. In addition to these differences, for all three species, changes in expression of important immune genes (including genes for IgM and IL-1β) were observed via qPCR, with the largest downregulation tending to occur in fish exposed to both oil and the pathogen *V. anguillarum*. Beta globin expression levels tended to be upregulated in early periods during the exposure and downregulated by the end of the exposure window. In addition to the suite of immune-related genes tested, levels of relevant proteins (including IgM and CYP1A) were assayed via ELISA to determine if changes in protein expression aligned with changes in gene expression, presenting a robust overview of how oil induces immune modulation in a variety of fish species from gene level to protein level. Taken together, these results provide an important link between findings from transcriptional-based studies of oil on immune function and actual, measured protein levels.

148 Exposure to a model thyroid inhibitor alters the immune response and pathogen resistance in male fathead minnows (Pimephales promelas)

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The sensitivity of the hypothalamic-pituitary-thyroid axis to environmental contaminants and the subsequent effects on growth and development has been well established for many endocrine-disrupting compounds. However, recent studies have suggested that thyroid hormones may play a role in the regulation of immune function. This study sought to determine the impact of propylthiouracil (PTU, a known thyroid inhibitor) on various aspects of immune function in the fathead minnow (*Pimephales promelas*). To achieve this, male fathead minnows were divided into two groups: a control and a PTU-exposed group. Following a 21 day exposure period, both groups were challenged with *Yersinia ruckeri*, and mortality was monitored for 14 days to assess pathogen resistance. Tissues (i.e. liver, spleen and kidney) were sampled at 8 and 72 hours post injection (hpi) for the assessment of immune gene expression and spleen index. PTU exposed males experienced significant decreases (~20%) in survival following pathogen challenge relative to controls. In addition, PTU-exposed males had a significantly lower mean spleen index (~15%) than controls following injections, suggesting that they had a reduced ability to elicit an immune response. At 8 hpi, PTU exposed males also experienced a significant decrease in the percentage of leukocytes relative to controls. Though not statistically significant, a 1.9-fold decrease in interleukin 1β expression and a 2.3-fold increase in myeloperoxidase expression were observed in the hepatic tissue of PTU exposed males at 8 hpi relative to controls. Finally, the expression of interleukin 11, a transcript involved in platelet production, was upregulated 3.2 and 56.4-fold in PTU exposed males at 8 and 72 hpi, respectively. Given that interleukin 11 would be upregulated in response to hemorrhaging associated with *Y. ruckeri* infection. This suggests that PTU exposed males experienced a more intense infection relative to controls. Overall, these results demonstrate that chemically induced thyroid disruption can suppress immune function and that the immune system may be a target for thyroid disrupting chemicals.

149 The immunome as a novel target for environmental chemicals: A case study with organochlorine pesticides

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Omics-based studies continue to shed light into the mechanisms of action of environmental contaminants. The immunome is defined as the set genes, proteins and metabolites that constitute and/or interact with the immune system. Field and laboratory transcriptomic studies conducted by our group in the largemouth bass (*Micropterus genus*) have revealed that organochlorine pesticides (OCPs) regulate the immunome. Largemouth bass (*Micropterus genus*) are apex predators that have a long standing history in Florida as a species faced with high pesticide exposure. Lake Apopka was contaminated with OCPs from a spill from the Tower Chemical Company in the 1960s. This event was compounded by runoff from agricultural lands on the north shore of the Lake that were used heavily for agriculture. While our past studies firmly established widespread reproduction impairment in bass following exposure to OCPs, recent studies point to the immune system as a preferential target. LMB placed into ponds in the Lake Apopka region for four months showed an altered immunome in the ovary and processes related to T cell suppression and leukocyte accumulation were differentially expressed. In follow-up laboratory studies, bass were fed OCPs (125 ppm p,p'-DDE and 10 ppm MXC) for ~2.5 months. Transcriptomics profiling in the ovary again revealed that gene networks related to leukocyte cell adhesion and platelet function were differentially expressed. Interestingly, immune-related networks were suppressed by OCPs, consistent with the immunosuppressive effects of OCPs reported in mammals. New approaches such as lipidomics have revealed alterations of cholesterol, sphingolipids, ceramides and glycerophospholipids in the liver of bass following OCP exposure, important because of the association between lipid-based molecules, inflammation, and immune suppression. These data demonstrate that immune health in bass may be impacted by OCPs and that interaction between the reproductive and immune systems should be considered in future studies studying latent effects of these persistent organochlorine pesticides.

150 Evidence for life-stage dependent immunotoxicity in the northern leopard frog

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Determining how contaminants, like polychlorinated biphenyls (PCBs), affect amphibian immune function may lead to improved understanding of amphibian population declines. *Lithobates (Rana) pipiens* tadpoles were orally exposed to environmentally relevant levels of PCB-126 (≤ 5 ng/g) through metamorphosis. To assess adaptive immune response, we immunized post-metamorphic frogs with keyhole limpet hemocyanin (KLH), and measured specific-KLH IgY (Trial I). To test whether life stage affected immune response, we ran two additional trials: Trial II) PCB-126 larval dietary exposure with primary KLH immunization during the larval stage followed by a booster immunization post-metamorphosis, and Trial III) both exposure to PCB-126 and KLH immunizations were administered after metamorphosis. When tadpoles were exposed to PCB-126, KLH-specific antibody responses were suppressed compared to the controls, however, this suppression was not apparent when animals were exposed only after metamorphosis. To assess innate immune response, we measured complement hemolytic activity in frogs. Larval PCB-126 exposure decreased hemolytic activity of frogs compared to unexposed frogs, but this suppression was no longer apparent when frogs were exposed only after metamorphosis. The study results demonstrate differences in immunotoxicity between anuran life stages, and suggests a carry-over effect of larval immune disruption due to PCB-126 exposure, which persisted through metamorphosis.

151 Exploring the ecological and evolutionary implications of pesticide exposure on disease risk in amphibians

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Pesticides and infectious diseases are two common stressors that regularly occur together in nature. Given the documented lethal and sublethal effects of each stressor on individuals, there is the potential for interactive effects that alter disease outcomes and pesticide toxicity. We conducted a series of studies exploring the interactive effects of pesticides and pathogens (trematodes and ranavirus) on larval amphibians using controlled laboratory experiments. We found that prior infection with ranavirus increased pesticide toxicity; median lethal concentration (LC50) estimates were reduced by 72 and 55% for carbaryl and thiamethoxam, respectively. We also found that prior pesticide exposure exacerbated ranavirus-induced mortality by increasing mortality rates. These results underscore the importance of incorporating order of exposure into research examining the interactions between pesticides and anthropogenic stressors. For trematodes, we examined how prior exposure to carbaryl influences the ability of larvae to resist infection. Larvae exposed to carbaryl had 61% higher parasite loads compared to control larvae. Moreover, we observed lag effects such that individuals placed into carbaryl-free water for 14 days and then exposed to trematodes retained 63% higher parasite loads than control larvae. Thus, pesticide exposure can reduce the ability of amphibians to resist parasite infections and these effects can persist weeks following exposure. Lastly, we explored how population-level variation in pesticide tolerance in wood frogs influences susceptibility to trematodes and ranavirus. For trematodes, we discovered that populations with high tolerance to carbaryl experienced lower loads than populations with low tolerance. For ranavirus, we found no relationship between tolerance and survival, but populations with high tolerance experienced higher viral loads than populations with low tolerance. Therefore, the evolution of pesticide tolerance was associated with susceptibility to parasites and pathogens. However, the patterns depended on the type of parasite, underscoring the complexity between pesticide exposure and disease outcomes. Collectively, our results demonstrate the need to integrate ecology, evolution, and toxicology to understand how natural populations cope with pesticides and their implications for disease risk.

152 Overview of the past, present, and possible future of aquatic and wildlife environmental immunotoxicology

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Environmental immunotoxicology began in the late 1970s through the last century via technology transfer from the burgeoning field of mammalian-based immunology and pharmacology/toxicology. Techniques quickly became adaptable and routine for non-mammalian animals, including phagocytosis and phagocyte activation, natural killer-like cell activities, basic aspects of complement activation, and ultimately the ability to quantify antibody responses. One of the more iconic assays of the time was the Jerne plaque assay that allowed for quantification of specific antibody secreting cells in a variety of animals. Following these seemingly dated approaches by modern standards, immunologists developed multiple fish and wildlife models for biomedical, aquaculture, and environmental health approaches. Through the 1990s and into the 2000s, the number of studies describing the effects of individual compounds and as mixtures in experimental systems and models increased dramatically. These studies were often extended to biomarker studies carried out in field-sampled organisms. Many studies focused on limited endpoints of immune function, and led to the concept of a tiered approach to immunotoxicity testing. The drawback to/of these approaches was several fold; namely the large financial expense and limited interpretation of results. As in many fields of science, "one finds what one looks for," and many innocently erroneous conclusions were assumed as gospel during the formative years of environmental immunotoxicology. Acute and chronic stress as a mode of action in environmental immunology was just being fully appreciated. Moreover, it was not until further development of assays examining multiple endpoints, pathogen susceptibility testing, and especially modern "omics" that we began to appreciate that "toxicity" could also mean polarization – the suppression of one arm, or direction of immunity in favor of a different direction. Polarization of lymphoid cells by select contaminants is driven by interactions of inducible transcription factors (e.g., the AHR, STATs, HIF1, Nrf2). This may explain why some contaminants are anti-inflammatory, while others promote inflammation. Contaminant-induced immune polarization by TCDD and other AHR ligands demonstrates one such mechanism. In this overview, we will present future directions of environmental immunotoxicology in understanding the effects of contaminants on immune systems in the context of organismal fitness.

Great Lakes Restoration Initiative – Occurrence and Effects of Contaminants of Emerging Concern – Part 1

153 Synthesis of Impacts of Chemicals of Emerging Concern on Fish and Wildlife Health: A cross agency collaboration

E. Murphy, USEPA / Great Lakes National Program Office

Through the Great Lakes Restoration Initiative, a collaborative work group of Federal Agencies and Academia has engaged in a series of research efforts and projects aimed at identifying and assessing Great Lakes chemicals of emerging concern (CECs), correlating CECs with land use and ultimately developing information and recommendations for resource managers to help evaluate threats to fish and wildlife populations for stocking purposes. In order to reduce and prevent adverse ecological effects to fish and wildlife, and associated negative impacts on related economic and recreational activities in the Great Lakes, the presence and deleterious effects of CECs must be better understood and appropriately managed. CECs of interest include an assortment of industrial chemicals (e.g., flame retardants), agricultural products (e.g., pesticides), materials from urban nonpoint source run-off (e.g., pesticides, plasticizers), and pharmaceutical and personal care products. Many CECs are currently unregulated by State and/or Federal water quality programs and information about the toxicity of CECs to fish and wildlife is generally limited, particularly when compared to regulated contaminants (e.g., legacy contaminants such as organochlorines). Information generated by this collaborative work group will help resource managers select stocking locations where expected deleterious impacts to fish and wildlife would be minimized. This presentation will provide an overview of the collaboration and will highlight the successes, challenges, and some results of the work.

154 Pesticide Presence in Great Lakes Tributaries and Comparison to ToxCast and Other Water Quality Benchmarks to Screen for Potential Biological Effects

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Results from evaluation of fifteen classes of trace organic chemicals (e.g. insecticides, herbicides, PAHs, pharmaceuticals) in Great Lakes tributary

monitoring during the first four years (2010-2013) of the Great Lakes Restoration Initiative indicated that pesticides (n=13) were a chemical class with potential to exert adverse impacts on aquatic life. A follow-on study was done in 2016 with 196 samples analyzed for pesticides (n=120) and pesticide degradation products (n=117) to define concentration, temporal patterns, and potential for adverse biological impacts in 16 major tributaries of the Great Lakes. Pesticide classes included herbicides, insecticides, and fungicides along with select degradation products. Relevance of pesticide exposure to aquatic life is being evaluated by comparison to traditional water quality benchmarks such as USEPA Aquatic Life Criteria and the USGS Health-Based Screening Levels for pesticides, as well as results from "high throughput" in-vitro biological assay results from the USEPA ToxCast program. Collectively, these three sources include available information for 207 of the 236 pesticide compounds monitored. Results are being examined to determine which tributaries had the greatest potential for adverse biological effects from pesticides, as well as prioritization of the most influential pesticides in terms of toxicity. These analyses are being used as part of the Great Lakes Restoration Initiative to focus current and future investigations that will help understand likely adverse outcome pathways in organisms, and to formulate possible remediation strategies. The contents of this abstract neither constitute, nor necessarily reflect, official USEPA policy.

155 USEPA bioeffects monitoring under the Great Lakes Restoration Initiative: Overview of efforts to assess the biological impacts of CECs

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Concern exists regarding the potential biological effects of contaminants of emerging concern (CECs) in the Great Lakes. CECs originate from multiple sources, including agriculture, wastewater effluents, and urban nonpoint sources. The Great Lakes Restoration Initiative (GLRI) Action Plan II has prioritized identifying and assessing the impacts of CECs on Great Lakes wildlife as a critical factor in protecting and maintaining Great Lakes ecosystems. As part of this commitment, efforts at USEPA Mid-Continent Ecology Division (MED) have focused on the development of bioeffects tools and approaches to identify potential effects from exposure to environmental mixtures. The approach involves deployment of caged, adult fathead minnow (*Pimephales promelas*) along with a composite water sampler, which collects an integrated water sample during the environmental exposure. Following in situ exposure, tissues are collected for targeted (i.e., steroid hormones, transcriptional effects) and untargeted (e.g., 'omics) analyses. Composite water samples are used for chemical characterization of the environmental mixtures through targeted analytical chemistry and for cell-based bioassays. This approach has been applied at multiple watersheds across the Great Lakes over the past six years, including Duluth-Superior Harbor, MN; Maumee River, OH; Detroit River, MI; and Milwaukee Estuary, WI. This work has further led to the development and refinement of predictive toxicology tools, comparing characterized chemical exposures to toxicity databases (CTDBase, ToxCast) to identify putative biological pathways impacted by CECs. The approach and developed tools will be detailed, focusing on observed effects and lessons learned.

156 Novel approaches for integrating chemistry, transcriptomics and physiology of caged fish to examine the impacts of contaminants of emerging concern

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The Great Lakes are impacted by the introduction of a large number of chemical of emerging concern at low concentrations in surface waters by wastewater treatment plants (WWTP). Here we present novel approaches for understanding the potential impacts of the individual components of complex mixtures using effects based monitoring and transcriptomics. We applied these approaches to assess the hypothesis that the discharge of chemicals by WWTP into Saint Louis Bay, Duluth, MN would cause estrogenic effects on fish exposed in the bay. To test the hypothesis, Fathead minnows were deployed in cages for 2, 4 or 8 days at three locations near two different WWTP plant discharge sites in the Saint Louis Bay, Duluth, MN and one upstream reference site. Surface water chemistry was determined from grab samples of water near deployed fish on days 2, 4 and 8. The biological impact of 51 chemicals detected in the surface water was determined using biochemical endpoints, in vivo and in vitro exposure activity ratios for biological and estrogenic responses, transcriptional pathway analysis, ovary–function gene set enrichment analysis, known chemical:gene interactions from Ingenuity Pathway Analysis (IPA) and Comparative Toxicogenomics knowledge bases, and analysis of the co-variance of ovary gene expression with surface water chemistry. Thirty-two chemicals were significantly linked by co-variance with expressed genes. While no significant impact on estrogenic endpoints was observed in male or female minnows, bisphenol A was identified by chemical:gene co-variation as the most impactful estrogenic chemical across all exposure sites. This was consistent with identification of estrogenic effects on gene expression, high bisphenol A exposure activity ratios across all test sites and historical analysis of the study area. Analysis of ovary transcriptomics also indicated that fish were exposed to chemotherapeutics, immunosuppressants and other therapeutics at one WWTP discharge site consistent with treatment of a hospital waste stream at that WWTP. Overall, this approach appears useful in examining the impacts of complex mixtures on fish and offers a potential route in linking chemical exposure to estrogenic effects that may reduce population sustainability. The contents of this abstract neither constitute nor necessarily reflect USEPA or USACE policy.

157 Mussel Watch and Metabolomics: Connecting exposure and effect in Great Lake bivalves

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The Mussel Watch Program (MWP) has used bivalves to monitor contaminant levels and environmental health since 1986. In 2009, monitoring of legacy contaminants was expanded to the U.S. Areas of Concern to support the Great Lakes Restoration Initiative (GLRI). In 2015, Under Action Plan 2 of GLRI, MWP together with other federal partners began to address contaminants of emerging concern and explore the use of effects-based indicators. Bivalves, as generally immobile filter feeders that bioaccumulate contaminants, are excellent representatives of local conditions and can be used as sentinel markers of both exposure and effect. Using metabolomics to study reproducible differences in metabolite levels between subjects can provide early indications of detrimental health effects. Coupled with contaminant loads and site condition information, we can evaluate subject exposure levels and their biological impact on

Great Lakes species. In this study, we analyzed the metabolomes of 480 caged dresissenid mussels, caged clams and in situ mussels collected from 12 sites on the Rouge River, the Detroit River and the Maumee River. A targeted metabolomics approach was used to measure the concentrations of 222 metabolites that included amino acids, biogenic amines, lipids, fatty acids, hexose and metabolites associated with energy pathways. Multivariate statistical analyses were performed on the resulting metabolomics data to identify relationships between geographical sites and to identify metabolites whose concentration levels increase or decrease between specific areas, species and time points. One such observation is that clams collected at Swan Creek and Perrysburg show metabolomic profiles more closely related to each other than to clams collected from any other Maumee River site. Further analysis identified 49 metabolites with statistically significant concentration increases or decreases in both Swan Creek and Perrysburg clams compared to control clams. Levels of alkylphenols, multi-residue pesticides, pharmaceutical and personal care products and metal concentrations have also been measured in bivalves from the same sites and integration of these results with the metabolite data is in progress. The ability to observe metabolite profiles distinct to geographical locations and to combine this information with contaminant level data provides a powerful tool to monitor this environment and to understand the biological impact of environmental changes.

158 Contaminants of Emerging Concern in tree swallows nesting on the Maumee River, Ohio

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A combined effort of USEPA, NOAA, USFWS, ACOE, and USGS evaluated contaminants of emerging concern (CECs) on the Maumee River, Ohio, in 2016. USGS collected tree swallow eggs, nestlings, and diet from six locations on the Maumee River; sediment, and aquatic invertebrates were also collected. The locations differed in relation to agricultural (up river) and industrial exposure (down river); two locations were associated with waste water treatment plants. Selected samples were analyzed for PCBs, PBDEs, PFCs, PAHs, pesticides, personal care products, metabolomics, and transcriptomics. Biomarkers were analyzed and included EROD activity, DNA damage, and thyroid hormone concentrations. Trends of environmental contaminants were associated with landscape (agriculture vs industry) and the proximity of water treatment facilities. Multivariate analysis identified significant differences among locations for all environmental contaminants. Of 141 personal care products, 8 were detected in 7 pools of nestling livers, 9 in 1 pool of nestling diet, and 19 in 6 sediment samples. Metabolomic profiles differed among locations. Biomarker and transcriptomic analyses are ongoing.

159 Ecological Hazard Assessment of Potential Impacts to Fish from Contaminant of Emerging Concern Exposures in the Great Lakes Basin

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An ecological hazard assessment (EHA) was performed to evaluate relative hazard to fish populations from exposure to a suite of contaminants of emerging concern (CECs) in the Great Lakes system. From 2010-2014, the U.S. Fish and Wildlife Service, together with the U.S. Geological Survey and other partners, coordinated to sample surface water at 25 sampling locations distributed throughout the U.S. Great Lakes Basin. There were multiple sampling sites within each location. All samples were analyzed for over 150 contaminants. The U.S. Fish and Wildlife Service derived ecotoxicological screening values (SVs) for assessing hazards to fish for 14 CECs commonly detected in surface water samples. The SVs represent CEC concentrations in water that bound our expectations for adverse effects in fish. Relative hazard scores were developed by comparing screening values against measured water concentrations at each sampling site. Both CECs and sampling locations were ranked with respect to their potential to impact fish populations at sampling locations, based on average hazard scores. Data gap analysis and hazard rankings provide the basis for prioritizing further research and natural resource or waste management actions. This presentation describes final screening values for 14 CECs, and provides an example to practitioners as to their potential applications in chemical hazard assessment. It expands on a preliminary EHA presented at the 36th SETAC NA meeting in 2015.

160 Mixtures of Contaminants of Emerging Concern common in Great Lakes tributaries reduce reproductive potential in wild and laboratory exposed fishes

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Analysis of nearly 500 surface water samples collected as part of the Great Lakes Restoration Initiative at 54 sites in tributaries of the Great Lakes Watershed confirmed the ubiquitous presence of Contaminants of Emerging Concerns (CECs) in anthropogenically impacted aquatic environments. Cluster analyses of commonly detected CECs in this data matrix suggests that the co-occurrence of approximately half of the CECs can be attributed to dichotomous urban or agricultural upstream land use. Mixtures found in watersheds with urban influences commonly contained steroidal estrogens, BPA, alkyphenols, pharmaceuticals and personal care products. Agriculturally influenced sampling sites contained herbicides and pesticides in addition to BPA and alkylphenols, but mostly lacked pharmaceuticals and personal care products. Almost 3,000 resident and caged sunfish (*Lepomis ssp.*) were collected from 27 of the 54 sampling sites and analyzed for indicators of stress associated with CEC exposure. In the presence of high aqueous CEC concentrations, glucose concentrations spiked in sunfish plasma and liver cells exhibited toxic stress response. Canonical correspondence analyses revealed that concurrent with indicators of toxic stress, biomarkers of reproductive potential declined. To further examine the population level consequences, fathead minnows (*Pimephales promelas*) were exposed in the laboratory for three generations to the empirically derived urban CEC mixture at three environmentally relevant concentrations. Subtle effects in the first exposed generation became more pronounced in subsequent generations with reduced growth and reproduction of second generation minnows and third generation juveniles lagging behind their control conspecifics in growth and in performing vital behaviors. Taken together, this integrated series of studies indicates that CECs in Great Lakes tributaries may impact fish population health and sustainability.

New Approaches to Ecological Risk Assessment – Bridging Adverse Outcome Pathways to Dynamic Energy Budget Models

161 Challenges in incorporating sub-organismal processes represented by quantitative AOPs into dynamic energy budget models

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In ecotoxicology research, two approaches have been gathering support because of how each tackles problems related to risk assessment. The adverse outcome pathway (AOP) framework is a "bottom-up" approach that links molecular initiating events to adverse outcomes that are relevant for predicting responses at higher levels of biological organization. Dynamic Energy Budget (DEB) theory offers a "top-down" approach that reverse engineers a modular characterization of the physiological effects of exposure to toxicants. We are co-PIs of a working group at the National Center for Mathematical and Biological Synthesis that is exploring the feasibility of using DEB models of individual organisms as a "pivot" connecting suborganismal processes to higher level ecological processes. AOP models quantify explicit molecular, cellular or organ-level processes, but do not offer a route to an integrated characterization of growth, reproduction, and survival. DEB models do describe these processes, but use abstract variables with undetermined connections to suborganismal biology. Several on-going projects aim to link DEB and quantitative AOP models by interpreting AOP key events as measures of damage-inducing processes in a DEB model. We report on the type and structure of data that are generated for AOPs. We also report on our progress on case studies that merge information collected for AOPs with DEB models, highlighting the challenges encountered by us – and other presenters in the session. One study focused on endocrine disruption in fish for which there are quantitative AOPs that integrate molecular, cellular and organ level responses to predict effects on reproduction. Connecting with a DEB representation required modifying the "standard" DEB model to include feedbacks that characterize the integrated effects of hormonal control mechanisms, thereby opening the way to connection with two previous qAOP models. Other studies focused on stressed freshwater zooplankton (*Daphnia*) and phytoplankton (*Chlamydomonas*). Here, one possible approach is to seek correlative connections between changes in DEB parameters and simultaneous transcriptomic or metabolomic responses. Finally, we discuss how the linkage of these two approaches can improve ecological risk assessment, with possibilities for progress in predicting population responses to exposure, but there still exists major challenges in extrapolating to ecosystems due to the absence of tested DEB theory at that level.

162 Connecting suborganismal and organismal responses using Dynamic Energy Budget Modeling and the ecological model species Fundulus heteroclitus

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Ecological Risk Assessment (ERA) is used to predict the ecological effects of stressors, typically using data collected on a limited number of laboratory species at the organismal level. Comprehensive and efficient ERAs depend on our ability to quantitatively extrapolate the effects of stressors, including toxic chemicals, under various environmental conditions, across levels of biological organization, and among species. Bioenergetics models, such as Dynamic Energy Budget (DEB), have been used successfully with many species to extrapolate from organismal- to ecological-level effects of multiple stressors including resource limitation, temperature variation and, more recently, toxic chemical exposures. In addition, recent ERA advances take advantage of the Adverse Outcome Pathways (AOP) framework to link sub-organismal mechanistic molecular information about toxic chemicals to organismal outcomes, increasing the potential to extrapolate effects across species. Here, we are exploring the use of molecular information and bioenergetics modeling (DEB modeling) within an AOP framework to quantitatively predict the ecological outcomes of multiple stressors, including combined chemical and food stress. Specifically, we are studying an ecologically-important species, *Fundulus heterclitus* (Atlantic killifish), with a sequenced and especially well-annotated genome and a long history of laboratory and field testing. We are developing killifish DEB models using abundant literature data and laboratory studies carefully designed to assess the interacting effects of temperature, resource levels, and dietary contaminant exposures. We are testing the system using model metabolic disrupting contaminants, specifically those whose AOPs include disruption of the thyroid hormone regulatory system, and measuring the effects of exposures on suborganismal- (transcriptomic or metabolomic), and individual-level (growth and reproduction) processes. In this presentation we will give an overview of our objectives and methods and also report preliminary findings fitting DEB models to data on starvation and recovery dynamics in killifish.

163 Hormone-driven energy allocation for egg loading added to a dynamic energy budget model to predict the effects of endocrine disruption

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Quantitative adverse outcome pathways (AOP) have been developed to predict the effects of endocrine disturbance on fish vitellogenesis using ordinary differential equation models to simulate the hormone dynamics underlying egg production. These vitellogenesis models are designed to predict egg production, and do not consider the entire energetic budget of an organism. Dynamic energy budget (DEB) models are complementary because they place the reproductive axis within the context of an entire organism and consider energetic tradeoffs between competing physiological processes. Thus AOPs linked to DEBs are better equipped to explore endocrine disruptors under various environmental conditions. Here we demonstrate how to link the mechanistic models of the hypothalmus-pituitary- gonadal axis that simulate vitellogenesis to a standard DEB model. In the standard DEB framework an organism allocates energy to a reproduction buffer which can be potentially converted to eggs. The strategies of handling the buffer are species-specific and are not explicitly specified. Using the concept of synthesizing units and autocatalytic processes, the reproductive energy flux and the estradiol- 17β – estrogen receptor complex combine to produce vitellogenin that can be used to predict egg production. We simulate scenarios of endocrine disruption within the DEB framework under multiple stressor situations, such as changes in food and temperature.

164 Daphnia as a model to advance Adverse Outcome Pathways

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There are many different approaches being used to advance Adverse Outcome Pathways (AOPs). For instance, omics technologies can provide mechanistic information on the effects of chemicals and can therefore help elucidate mechanisms of toxicity. In recent times, efforts have been focused on developing measurable linkages between key events (KEs) in order to establish quantitative AOPs (qAOPs). Different systems and modeling techniques are being considered and applied to develop measurable KE Relationships (KERs) such as flux balance analysis, reverse toxicokinetic models, physiologically-based models. In particular, the linkages between qAOPs and dynamic energy budgets could improve risk assessment by tapping into 30 years of established metabolic theory and to constrain qAOPs within realistic energetic demands of organismal function. Here, we explore the use of dynamic energy budget (DEB) models, which describe the assimilation of energy by an organism and its allocation to growth, development, maintenance and reproduction at all life stages, in order to connect information at different levels of organization. Linking the DEB models to AOPs will eventually allow for the screening of thousands of potential stressors on many species, but will require empirical data on a few species to validate models and identify appropriate assays to use for risk assessment methods. In the present study, we identified critical data gaps that are needed to link molecular responses to dynamic energy budget processes related to growth, reproduction and reserve dynamics. We used *Daphnia magna* as a model organism because it has a sequenced genome, it can easily be used to obtain population and community level data in a laboratory, and it is the focus of several AOP and DEB studies already (though no study has tried to link AOP and DEB). We exposed individual daphnids to a complex mixture of contaminants found in coal ash, and evaluated contaminant body burdens at key life history and molt cycle stages as well as parameters relevant for DEB modeling (weight, length, reproduction, etc) in daphnids exposed to different food and contaminant treatments. We are also exploring the use of gene expression to inform the DEB model. These experiments represent a critical first step to bridging the gap between molecular-level responses and larger scale, longer-term impacts at the population and ecosystem level.

165 Integrating dynamic energy budgets with adverse outcome pathways: A case study with mitochondrial dysfunction leading to impaired vitellogenesis

D.A. Dreier, N.D. Denslow, C.J. Martyniuk, University of Florida / Physiological Sciences

Vitellogenin is an egg-yolk precursor molecule synthesized in the liver of oviparous species. This molecule is an important reproductive biomarker in ecotoxicology, and there are several individual-based models that have correlated female vitellogenin levels with population viability. Decreases in vitellogenin production are detrimental to fish populations, and these effects can be produced through several endocrine modes of action, including aromatase inhibition, estrogen receptor antagonism, and androgen receptor agonism. However, based upon our ToxCast computational analyses, there are many substances that may not interact with these endocrine pathways, but still decrease vitellogenin levels through other mechanisms, such as mitochondrial dysfunction. Thus, the objective of this study was to strengthen the adverse outcome pathway for impaired vitellogenesis by quantifying the relationship between mitochondrial dysfunction and vitellogenin production in the liver. Our hypothesis was that mitochondrial dysfunction and decreased ATP:ADP ratios in the liver would reduce vitellogenin production, as energy would be prioritized for somatic maintenance. To test this hypothesis, we developed an ex vivo approach to measure mitochondrial bioenergetics in fish liver. In specific, we optimized a precision-cut liver slice assay in largemouth bass to measure mitochondrial bioenergetics in the Seahorse XFe24 Extracellular Flux Analyzer. This assay, entitled the Bass Liver Slice Assay (BLiSA), utilizes three mitochondrial inhibitors (oligomycin, FCCP, sodium azide) to measure specific mitochondrial parameters, including basal respiration, ATP production, maximal respiration, and spare capacity. Liver slices 200 microns thick and 2 mm in diameter produced the most consistent oxygen consumption rate (OCR) data, with basal respiration at 160 pmol/min OCR. We will expose liver slices to established mitochondrial toxicants (2,4-dinitrophenol, paraquat) and measure mitochondrial parameters ex vivo, which will be correlated with vitellogenin mRNA and protein levels. Results will be integrated into a dynamic energy budget model to advance quantitative predictions of vitellogenin production and reproduction dysfunction in female fish.

166 Integrating lethal and sublethal toxic impacts on an estuarine amphipod within a dynamic energy budget modeling framework

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Ecotoxicological risk assessment studies are necessarily confined to narrow subsets of relevant species, toxicants, exposure conditions, levels of impact, and so on. This problem is often exacerbated by the relatively wide margins of error that typically accompany data and observations from those studies, especially at the higher levels of biological organization. Therefore, ecotoxicological risk assessment critically depends on sound methodologies that translate and integrate the results and observations from toxicity studies along a multitude of axes, including those defining experimental conditions (e.g. exposure time), a horizontal one relating endpoints at the individual level (e.g. growth and reproduction), and a vertical one for making projections - or educated guesses - across levels of biological organization. Dynamic Energy Budget (DEB) models are uniquely equipped to translate and integrate information obtained from toxicity studies along many of those axes, in particular the horizontal axis and those setting experimental condition; these models also establish a link between the individual and population level. DEB models describe the rates at which organisms acquire resources and spend the energy and nutrients therein for general processes, such as growth, maintenance and reproduction. In this presentation, we develop a DEB model to investigate the impact of copper nano- and micro-pesticides (CuPro and Kocide), ionic copper and a neonicotinoid pesticide (imidacloprid) on the estuarine amphipod *Leptocheirus plumulosus* in short term starvation and longer term feeding experiments. The toxicokinetics and toxicodynamics of these compounds in the amphipods were analyzed with time-resolved data on bioaccumulation, motility, biomass accrual or turnover, survival and cannibalism. Some of the data were collected at the individual level, whereas others concern the whole population (initially 20 per container). In analyses of the latter, the population was treated as a super-organism. The data show that the impact of copper depends little on the types of compound administered, which suggests that nano and micro copper are readily ingested and assimilated from the gut. Furthermore, the DEB model in this study connects toxic impacts on different 'endpoints' at several time points and at two levels of organization, which demonstrates its ability to translate and integrate information from toxicity studies along a variety of axes.

167 Modeling concurrent nutrient and toxicant stressors under dynamic energy budget theory

A. Peace, Texas Tech University / Mathematics and Statistics; G.D. Mayer, Texas Tech University / Environmental Toxicology

There is increasing evidence that organisms experience interactive effects of contaminant stressors and food conditions, such as resource stoichiometry and nutrient availability. We are developing and analyzing a series of empirically testable and robust mathematical models of population dynamics subject to concurrent stoichiometric and contaminant stressors. The dynamic energy budget (DEB) theory provides the framework to deal with simultaneous toxicant stressors and stoichiometric constraints. We present a case study model of *Daphnia* under toxicant stress predating on algae of varying nutritional quality (carbon: phosphorus composition).

168 Advancing TKTD modeling of developmental toxicity: Quantifying glutathione dynamics in the developing rat embryo

K. Veltman, Y. Ahmad, University of Michigan / Environmental Health Sciences; C. Harris, University of Michigan School of Public Health / Environmental Health Sciences; O. Jolliet, University of Michigan / Environmental Health Sciences

A quantitative understanding of glutathione:glutathione disulfide (GSH:GSSG) redox dynamics is essential to develop ToxicoKinetic-ToxicoDynamic models for developmental toxicity. The GSH:GSSG balance affects a variety of basic cellular functions (e.g. proliferation, differentiation and apoptosis) that are critical to ensure a normal embryonic development. In addition, many teratogens elicit their toxic action by perturbing this redox balance causing malformations, behavioral deficits and/or embryonic lethality. Precise control of the GSH:GSSG redox balance is vital for the developing embryo, but a quantitative, systems-level understanding of conceptal redox dynamics is presently lacking. We develop a dynamic mass-balance model for overall GSH metabolism in the organogenesis stage rat conceptus that accounts for the mass-fluxes of GSH precursor uptake, utilization, turnover, and enzyme kinetics.

Two initial challenges were confronted: First, rapidly growing embryos (EMB) can create a considerable 'growth dilution' if not accounted for, and second, GSH:GSSG dynamics are spatially-segregated where the visceral yolk sac (VYS) and EMB proper retain their distinctly separate redox dynamics. We therefore obtained consistent time-course data of relevant growth parameters in rat whole embryo cultures for VYS surface area, size, volume, and the concentrations of GSH, GSSG, cysteine (Cys), and cystine (CySS) in the VYS, yolk sac fluid (YSF), amniotic fluid (AF), and in the EMB proper. We observed significant exponential growth for the VYS surface area (r^2=0.97), VYS protein synthesis (r^2=0.82) and EMB protein synthesis (r^2=0.89). Our results show that concentrations of GSH and GSSG (nmol/mg protein) remain remarkably stable during the organogenesis period in VYS and EMB, indicating that GSH:GSSG is maintained at steady-state levels in both tissue compartments, albeit at different levels. A mass-balance model consisting of 21 coupled differential equations describing transport fluxes, enzyme kinetics, biosynthesis and growth dilution of 8 metabolites (incl. GSH, GSSH, Cys, CySS) in 3 compartments (VYS, YSF+AF, EMB) was developed. Results show good overall agreement between model predictions and measured data. Current research focusses on further model development using perturbation experiments. Our model forms an essential basis to assess adverse outcome pathways (AOP) and to develop mechanistic TKTD models for developmental toxicity caused by oxidative stress.

Conflicts of Interest and Normative Science – Is It a Problem in Environmental Science?

169 Normative Science: What is it, how pervasive is it in the environmental sciences, and should we be concerned?

T. Canfield, USEPA / ORD NRMRL GWERD; V. Forbes, University of Minnesota / Ecology, Evolution & Behavior; C. Lehman, Dow Chemical Company / Toxicology and Environmental Research and Consulting

Normative science (sometimes referred to as white hat bias) has been defined by some as a type of information that is developed, presented, or interpreted based on an assumed (usually unstated) preference for a particular policy or class of policies. As ethical scientists, we are trained to let data and facts guide our conclusions, as well as help define future hypotheses. However, as humans we have values, beliefs, morals and convictions that often make remaining unbiased challenging, even if we don't recognize it. We potentially see this bias when we initially choose what area of research to pursue versus those we are not interested in pursuing. But what is normative science? Is normative science a part of the environmental sciences and the research that is conducted? If so how pervasive is it? Is it only a part of one sector (academia, government, business, NGO) or does it manifest itself across multiple, if not all, sectors? This presentation will explore the nature of normative science in environmental science, what it might look like, where it may be prevalent and look to evaluate how pervasive is it (if at all) and how much of a concern it might be currently in the environmental sciences.

170 Don't copy Rachel; that is no way to give science advice for environmental policy

P. Calow, University of Minnesota / ORED

Judgments are an unreliable source of environmental policy because they are often wrong. The scientific process provides a more reliable foundation for policy being based on evidence that can be checked. So environmental advocates, like Rachel Carson, who mix judgement with evidence in interpreting the uncertainties in the links between causes and adverse ecological effects, and in arguing for particular remedial policy options, potentially undermine the strength of science as a source of advice in informing environmental policy. She apparently got it right – but most of us don't. And if, in this post-factual world, the judgements of scientists are confused with the outcome of the science process then we should expect that science advice is treated no differently from political judgements in the policy arena. This paper will outline this argument, show how it relates to some deep philosophical issues connected to Hume's *is* and *ought*, and argue that if it is to maintain its credibility the environmental science process has to recognize and come to terms with these challenges. More specifically for SETAC, this means that uncertainties in the links between exposures and effects should always be transparent and dose-response relationships, core to our discipline, should be treated as delimiting policy choices rather than as defining specific outcomes.

171 Navigating Values in Environmental Science

K. Elliott, Michigan State University / Lyman Briggs College / Fisheries & Wildlife / Philosophy

Building on recent scholarship in the philosophy of science, this talk will argue that social and ethical values have a number of legitimate roles to play in environmental science. First, drawing on my recent book, *A Tapestry of Values: An Introduction to Values in Science* (Oxford University Press, 2017), I will highlight the range of judgments involved in doing environmental science—including judgments about what topics to study; what questions to ask about those topics; what characteristics are most desirable when developing theories or models; how to respond to scientific uncertainty; and what terms, frames, and categories to use for describing results. These judgments are typically not settled by empirical evidence, and depending on how they are made, they often serve some social or ethical values over others (even if scientists are not purposely trying to promote particular values when making the judgments). For example, when modeling the future effects of climate change, scientists are forced to make value-laden choices about what phenomena to model, how far into the future to extend the models, whether to disaggregate the effects on particular geographical regions or socio-economic groups, how to communicate about discrepancies in the results from different models, how heavily to discount economic costs over time, and so on. I will argue that the best way to address these value-laden judgments is typically not to try to exclude social and ethical values from science but rather to make these judgments more transparent and to subject them to critical deliberation. Given that many of these judgments cannot be settled by the empirical evidence, efforts to exclude value influences are likely to backfire and result in surreptitious value influences that are not subject to adequate discussion. Moreover, social and ethical values are relevant to many of these judgments (e.g., choosing what topics to study, what questions to ask, and how to communicate results). I will argue that the scientific community ought to address values by fostering an array of strategies for highlighting and deliberating about them. This can include efforts to improve peer review, strengthen government regulatory institutions and advisory bodies, foster greater diversity among scientists, promote engagement between scientists and stakeholder groups, and create better policies for acknowledging and addressing particularly significant conflicts of interest.

172 Case Studies Indicate that Tools Currently Available to Assure Quality and Integrity of Federal Government Science Are Inadequate

A. LeHuray, Chemical Management Associates LLC; J.W. Conrad, Conrad Law & Policy Counsel; D.A. Kanter, Swanson, Martin & Bell, LLP

The National Academies have recently emphasized the critical importance of effective programs to ensure the quality and integrity of research conducted under the federal government's auspices in order to limit the impact of White Hat Bias and publication of normative science. This is particularly true for agencies that do not require scientists to use a disclaimer for approved publications, since local, state, and federal government officials, as well as news media and the general public, often erroneously conclude that papers authored by scientists employed by federal agencies represent the opinion of the US government. Yet recent examples show the inadequacy of administrative mechanisms currently operative within federal agencies, such as Information Quality Act (IQA) guidelines, peer review processes, and scientific integrity programs. When questions arise after publication, administrative tools available to the public have also been shown to be of limited value in correcting the scientific record. These include IQA guidelines, scientific integrity programs, and the Freedom of Information Act. Until these mechanisms can be made more reliable, affected parties must rely on extra-governmental mechanisms to request raw data, model code and parameters, and other underlying information, and to request agencies to correct information. These mechanisms include publication of public comments or letters to the editor and post-publication peer review vehicles such as PubPeer and PubMed Commons.

173 Addressing White Hat Bias: Lessons from Environmental Litigation

K.R. Palmquist, A.M. Morrison, Exponent / EcoSciences; M. Edwards, Exponent / Environmental and Human Health Consulting

Litigating complex, science-based issues requires experts from academia, government, and industry to testify about the relevant issues. These experts, in turn, often rely on published science to support their opinions. While legal representatives are tasked with defending and advocating for their clients, scientists and scientific experts are expected to maintain ethical impartiality. Given the tension between advocacy and scientific impartiality, a rigorous review of science, where impartiality is not presumed but repeatedly tested and critiqued, is essential. In *Daubert v. Merrell Dow Pharmaceuticals*, the United States Supreme Court opinion provides standards for the use of science and scientific experts in a court of law, focusing on both scientific validity and proper applications of methodologies and approaches. This decision both set criteria for inclusion of scientific evidence and provided a means for challenging flawed or biased science and experts. Although these criteria were developed to prevent reliance on pseudoscience in the court of law, they can also be useful in identifying cases of normative science that can arise from personal biases or desired outcomes. Examples of normative science will be presented along with the procedures for identifying them. In addition, the use of *a priori* conceptual frameworks, such as those used to support environmental causal analyses, will be presented, as these can be invaluable tools for maintaining impartiality and minimizing the impact of cognitive biases on scientific work.

174 Science Integrity, Publication Bias and Normative Science in Ecotoxicology: A Role for SETAC

P.D. Guiney, University of Wisconsin / Molecular and Environmental Toxicology Center

There is an increasing consensus among academic, government and industry scientists that a more systematic and unambiguous approach should be taken to address issues of scientific integrity. There continue to be reports of high profile studies of questionable practice which have led to the retraction of peer-reviewed publications. Historically many of these have come from the biomedical literature, however, the field of ecotoxicology is certainly not immune to such dubious practices (see "Science paper on microplastics in larval fish retracted" C&EN May 15, 2017). In 2015, the SETAC World Council voted to make Science Integrity a new initiative for us to address as part of our Long Range Planning program. Previous reports have shown that on average about 2% of scientists conduct fraud but, closer to 34% are involved in questionable research practices [QRP] (PLOS May 29, 2009). Surveys have shown that the most common QRP is not to publish a valid study which is a form of bias itself. Ecotoxicology like other sciences is not value-free. Even self-disciplined scientists carry some bias from cultural absorption, heuristic problem solving and their own personal experiences. However, there is also an expectation that science should be policy neutral. Because every study builds on insights produced by prior studies, the efficiency of the scientific process can be significantly impacted if published literature includes studies that report misleading results (intentional or unintentional). Similarly, scientific efficiency can be compromised if failures to produce findings are misdiagnosed as failures to reproduce earlier studies when in fact they are failures of earlier findings to generalize. When we think about the interface between science and policy making, there is a form of science that is infused with policy preferences. Such science may be labeled "normative science" and it is potentially a devious corruption of traditional science. Although normative science can represent clever policy advocacy, it has become a serious problem in our society. Misuse of science has become increasingly common in ecotoxicology and chemistry and this has the effect of undermining our science and the confidence in scientists. We need to develop a conceptual framework for considering scientific integrity in SETAC as an extension of our personal integrity and, encourage a self-correcting culture of scientific rigor, appropriate transparency and critical review.

175 Peter Chapman Energized Our Science Like Few Have: Dr. Weight-of-Evidence

G. Burton, University of Michigan / School of Natural Resources & Environment; R.J. Wenning, Ramboll Environ

Peter Chapman was to present at this time, but sadly passed from our world on 26 September 2017. We have the honor of reflecting on his contribution to science and SETAC, including his presentation intended for today. His career in ecotoxicology and chemistry began more than 30 years ago. His first big idea was the "Sediment Quality Triad," now internationally recognized. He challenged the users of science, throughout his career, to get it right - always taking the high-road of science rules without bias. As he entered the final phase of his career he reflected on the "big picture" and the challenges of the tripartite SETAC approach. Each of us is beholden to our employer and biases are hard to remove. His final presentation message may have been his most poignant for SETAC members and all environmental managers.

176 Scientific Integrity Issues in Environmental Toxicology

C. Mebane, USGS / Idaho Water Science Center; A. Fairbrother, Exponent, Inc. / EcoSciences; T. Augspurger, US Fish and Wildlife Service / Ecological Services; T. Canfield, USEPA / ORD NRMRL GWERD; W.L. Goodfellow, Exponent, Inc. / BioSciences Practice; P.D. Guiney, University of Wisconsin / Molecular and Environmental Toxicology Center; A. LeHuray, Chemical Management Associates LLC; L. Maltby, The University of Sheffield / Animal & Plant Sciences; D.B. Mayfield, Gradient; M. McLaughlin, University of Adelaide and CSIRO Land and Water; L.S. Ortego, Bayer CropScience / Environmental Toxicology and Risk Assessment; T.H. Schlekat, SETAC; R.P. Scroggins, Environment and Climate Change Canada / Biological Methods; J.P. Sumpter, Brunel University / Institute of Environment Health and Societies; T. Verslycke, Gradient

In 2015, the Society of Environmental Toxicology and Chemistry (SETAC) formed a new *ad hoc* subcommittee with collaboration from all three major sectors of the Society (academia, business, and government) to address how the membership is aware of and adheres to high standards of ethical behavior in scientific conduct and discourse. The charge for the group was to review current practices and develop new approaches by which SETAC can: (1) ensure scientific objectivity in review, editing and text of all its publications; (2) encourage objectivity in presentations at SETAC-sponsored meetings and workshops; (3) provide continuing education to SETAC members about scientific integrity; and, (4) join in the global conversation about scientific bias, ethics and transparency. Per Charge 4, we will discuss several scientific integrity issues relevant to Society members including navigating conflicting interests in academic-industry collaborations, and improving rigor, reproducibility, and transparency in the publication process.

Assessing the Role of Contaminants in the Decline of Prairie Complex Pollinators

177 Translating Risk Assessment Science into Risk Management Actions on a Prairie Landscape Scale

D. Warburton, US Fish & Wildlife Service

Risk assessment science is typically developed on a species-specific scale to produce dose-response curves with the (sometimes presumed) goal of informing risk management options. Even while more and more toxicity data continue to be generated, and exposure scenario models continue to become more refined, concerns about the landscape-scale impacts of pesticides and other contaminants are more prevalent than ever. These concerns are especially applicable to a wide variety of pollinators across the prairie systems of North America. This presentation will explore the challenges of developing risk assessment science that is relevant to land use and regulatory decisions affecting the quality of prairie systems, and will offer recommendations for both risk assessment science and risk management actions to help reduce the threat of pesticides and other chemicals to prairie pollinators.

178 The effects of ingested aqueous aluminum on floral manipulation and foraging pace in two subspecies of honey bee (Apis mellifera spp.)

A. Chicas-Mosier, Oklahoma State University / Integrative Biology; C. Dinges, S. Alvarado, C.I. Abramson, Oklahoma State University / Psychology

Pollinator decline is of international concern because of the economic services these organisms provide. Often, decline is attributed to toxicants, habitat fragmentation, and parasites. Applied and in-soil toxicants such as metals can be taken up by plant tissues and then distributed to pollinators. Such distribution occurs with the metal aluminum especially in acidified or Bauxite mined areas. A free-flying artificial flower manipulation apparatus was used to understand how a low concentration of aluminum (20 mg/L Al) may affect the pace, color fidelity, and foraging behaviors of two subspecies of honey bees (*Apis mellifera mellifera* and an *Apis mellifera mellifera/ scutellata* hybrid) in the United States and Puerto Rico, respectively. The results in the United States show a single dose of aluminum immediately affects the floral decision making of honey bees potentially by altering sucrose perception, this is presented as sustained color fidelity despite better options as well as fewer total cap interactions by exposed bees. Aluminum does not appear to disorient bees as it does not affect pace. We conclude that aluminum exposure may be detrimental to foraging behaviors and potentially to other ecologically relevant behaviors.

179 The role of pesticides drift in prairie remnants in the disappearance of threatened and endangered butterflies

E. Runquist, Minnesota Zoo / Conservation

Prairies are home to a diverse assemblage of butterflies, but many have declined dramatically in recent decades. Indeed, two prairie endemics, the Poweshiek skipperling (*Oarisma poweshiek*) and Dakota skipper (*Hesperia dacotae*), are now listed respectively as U.S. Threatened and Endangered species, despite formerly being predictable and widespread across Upper Midwest prairies. Many factors have likely contributed to their declines at regional and local levels, but a key hypothesized driver to explain their recent range-wide collapses is insecticide drift from adjacent agricultural operations. The Minnesota Zoo and U.S. Fish and Wildlife Service have been partnering to collect data on the extent of pesticide drift into native prairie remnants that house(d) these listed butterflies. Grass and soil samples collected from along prairie - agriculture edges and from prairie interiors in late spring and late summer have been analyzed for residues of a broad range of pesticides. In late spring, pesticides (primarily the herbicide atrazine) have been found infrequently, and largely only along agricultural edges. However, three broad-spectrum insecticides (chlorpyrifos, lambda-cyhalothrin, and bifenthrin) applied against economically-damaging pest soybean aphids have been found at all five sampled prairies in late summer. These three insecticides have been recorded commonly in both edge and prairie interior points, sometimes at high levels (range of 5 to 275+ parts per billion), including inside designated critical habitat for the federally protected skippers. The increase in applications of these insecticides correlates with the range-wide collapses of imperiled prairie butterflies. New controlled laboratory toxicity studies are underway to determine the biological consequences of the range of insecticide exposures observed in the wild to caterpillars of grass skipper butterflies. Results from this work will inform risk assessments and regulatory mechanisms.

180 Minnesota Department of Agriculture, Pesticide Spray Drift Complaint Reporting Procedures and Actions

R. Mann, P. Haiker, K. Middendorf, J. Stamper, Minnesota Department of Agriculture

Pesticide spray drift can occur when pesticide particles move through the air away from the target site of application at the time of application or soon after. Even if pesticides are applied carefully drift can occur and pose risks to the human health and the environment. The Minnesota Department of Agriculture (MDA) is the state agency responsible for enforcing state and federal pesticide laws and investigating pesticide drift related complaints and other illegal use or misuse of pesticides. Investigations typically occur when a formal complaint is made to the MDA. The MDA pesticide drift or misuse investigations typically begin with a visit to the application site and the property that was drifted on to obtain vegetation, soil, water, pollinator or other relevant samples for lab analysis. The MDA inspectors also gather information on application records, maps, weather records, statements, and product labels. Once the investigation is complete, the MDA reviews the evidence to determine whether a violation has occurred and what action is appropriate. If the evidence establishes that a violation has occurred, the MDA has the authority to penalize the violator under state/federal laws. Depending upon the severity, penalties can range from a warning letter to monetary fines to license revocations. Depending upon the complexity, a drift complaint may take several months to determine whether a drift violation has occurred. The MDA receives approximately 150 pesticide-related complaints per year. Approximately 66 percent of reported complaints result in an investigation.

181 Method for estimating pesticide exposure for honey bees and considerations for estimating exposures to other species of bees and insects

J. Housenger, USEPA / Office of Chemical Safety and Pollution Prevention; K.V. Garber, M. Wagman, USEPA / Office of Pesticide Programs Environmental Fate and Effects Division

The U.S. Environmental Protection Agency (USEPA) uses a tiered approach to assess pesticide risks to bees. This approach is largely dependent on honey bees (*Apis mellifera*), which are important to USEPA's protection goals (i.e., pollination services, hive products, biodiversity). Pesticide risks to other bee species are also of concern, as one of the protection goals involves pollinator biodiversity. At tier I, risk is assessed by comparing estimated exposures via contact and ingestion of contaminated pollen and nectar to laboratory-based toxicity data for honey bees. Exposure is assessed using the BeeREX model, which calculates pesticide exposures from honey bee food consumption rates and estimated pesticide concentrations in pollen and nectar. Contact exposure is assessed for adult bees directly sprayed while foraging on treated fields. In order to apply this method to other species of bees and insects, it is necessary to understand whether 1) the major exposure routes used for honey bees (contact, diet) are representative of major routes for other species, and 2) the magnitude of estimated exposure is protective of other species. In cases where major exposure routes for non-*Apis* species are not assessed or estimated exposures for honey bees are not protective for non-*Apis* bees, it may be necessary to develop methods for assessing exposures to other species. This presentation provides an overview of the methods used to assess pesticide exposures to honey bees and discusses considerations for leveraging existing methods to develop approaches for assessing exposure to solitary and social non-*Apis* bees and other beneficial insects. We discuss how this approach was used in recent USEPA risk assessments to characterize potential risk to non-*Apis* bees and other insect pollinatorsfrom environmental exposures to pesticides. Arthropod species information:Honey Bees (*Apis mellifera*), non-*Apis* bees (e.g., *Bombus* spp.; *Osmia* spp.; *Megachile* spp; *Nomia* spp; *Meliponini* spp.), butterflies (*Lepidoptera*)

182 Landscape Scale Estimates of Monarch Butterfly (Danaus plexippus) Population Responses to Insecticide Exposure in an Iowa Agroecosystem

S.P. Bradbury, T. Grant, Iowa State University / Natural Resource Ecology and Management; N. Krishnan, Iowa State University / Entomology

Current monarch conservation practices discourage placement of habitat within 38m of agricultural fields that use pesticides, despite potential net benefits of additional habitat sites. Foliar insecticide use to control late season soybean aphids (*Aphis glycines*) or early season fall armyworms (*Spodoptera frugiperda*) in fields with cover crops could expose monarch larvae on milkweed near crop fields, but potentials effect on adult recruitment at the patch and landscape scale are unknown. We generated instar-specific 96-hour mortality dose-response curves for beta-cyfluthrin, chlorpyrifos, imidacloprid, thiamethoxam and chlorantraniliprole. Using EPA's AgDRIFT model, we estimated spray drift exposure at distances up to 38m from treated fields for aerial and ground applications. For aerial applications, predicted percent mortality for 1st instars ranged from 100% to 44%, 100% to 7% and 100% to 1% at the edge of field, 15.24m and 38m away from treated fields, respectively. For 5th instars, percent mortality ranged from 100% to 0%, 97% to 0% and 82% to 0%. In general, predicted mortality rates were higher for beta-cyfluthrin and chlorantraniliprole and lower for thiamethoxam and imidacloprid. To assess monarch population responses at a landscape scale we created a GIS landscape layer of areas within 38m of crop fields in Story County, Iowa. This layer was incorporated into a spatially-explicit agent-based model that simulates adult monarch movement and egg-laying in 17 different habitat classes. A projection model subsequently estimated survival from egg to adult. Estimates of adult recruitment in the County were derived under three different scenarios: a) no milkweed within 38m of crop fields; b) milkweed patches placed within 38m of crop fields, with no insecticide exposure; and c) milkweed patches placed within 38m with spray drift insecticide exposure at varying distances downwind; mortality dose-response curves were used to estimate decreased larval survival due to spray drift exposure. In future studies, the projection model will incorporate larval mortality rates based on systemic insecticide uptake in milkweed downslope of fields planted with corn or soybean treated-seeds. Estimates of landscape-scale adult recruitment under varying spatio-temporal pest management scenarios provide the means to evaluate monarch conservation costs and benefits of establishing habitat in areas potentially exposed to insecticides.

183 Prospective Methods for Characterizing Likelihood of Pollinator Protection Resulting from Programmatic Conservation Initiatives

D. Perkins, Waterborne Environmental, Inc.; A. Schmolke, Waterborne Environmental, Inc. / College of Biological Sciences; F. Abi-Akar, A. Jacobson, Waterborne Environmental, Inc.

Over 4,000 species of native bees are responsible for crop pollination activity in the United States, the majority in solitary nests. County-, State-, and Federal-scale initiatives and programs have been put in motion toward programmatic protection of pollinators. Programmatic initiatives tend to focus on habitat creation, preservation, or restoration and should be accounted for in conservation efforts to protect pollinator species. These habitat initiatives may be a simple means to rapidly respond to pressures to implement protection measures, but may be less impactful or less appropriate for certain species of pollinators than others. A methodology for evaluating programmatic conservation initiatives and associated impact on pollinator protection is warranted and would require more specific identification of species that are the recipients of protection. The specific characteristics and requirements of the identified species should be addressed. Difficult and important discussions about cost-benefit and likelihood of protection success may be more fruitful if a common methodology is followed. We present preliminary methods that benchmark characteristics of land use change/management and pollinator life history features through programmatic conservation initiatives that yield the most benefit for pollinator protection. Land use change, prompted by potential conservation efforts, is systematically compared to focal species' requirements according to their life history traits and habitat requirements. As an example, we use the Rusty Patched Bumble Bee (*Bombus affinis)*, a species recent listed as endangered, as a test case for benchmarking potential protection through the introduction of different conservation initiatives, such as creation of conservation reserve program land, pollinator corridor creation, cover crops, and integrated pest management.

184 Discussion - Moving forward in protecting prairie complex pollinators

N. Golden, US Fish and Wildlife Service / Ecological Services; S. Warner, US Fish and Wildlife Service / Environmental Contaminants Program

For this session, we bring together experts from state agencies, federal regulatory and management agencies, and researchers from academia and non-governmental organizations. Session chairs will facilitate a discussion to further explore issues presented related to assessment and management of prairie pollinator species. Do regulatory mechanisms generated at the state and federal levels provide an adequate framework for local management practices? Is there enough flexibility to allow for the incorporation of knowledge at a more refined level (e.g., species-specific or geographically)? How do current research findings influence current regulatory and management practices? And is research being conducted in a manner to facilitate the transfer of findings into these practices? Finally, how can stakeholders better come together to address species and habitat loss across the landscape? Speakers will be invited to participate in a discussion of these topics and others, and input from audience members will be encouraged.

The Ecotoxicology of Elevated Major Ion Mixtures in Fresh Waters

188 Can Changes in Transepithial Potential Explain Major Ion Toxicity?

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Salinization of freshwater ecosystems is a rising environmental concern to which multiple anthropogenic activities contribute, each with its own unique mixture of major ions. As a result, development of broadly applicable environmental standards for regulation of freshwater salinization is complicated. There have been a number of proposed approaches for regulating mixtures of major ions including conductivity-based standards, empirical toxicity relationships, and mechanistic models. One mechanistic approach is based on the premise that the toxicity of major ion mixtures can be predicted as a function of changes in organism transepithelial potential (TEP), i.e., a given change in TEP is associated with toxicity regardless of the major ion concentrations and composition needed to elicit that change. Initial model development involved predicting organism TEP and fitting ion permeability coefficients such that variance in toxicity predictions were minimized. This approach allowed for toxicity predictions across a wide range of waters with varying major ion concentrations and compositions. While these results are promising, there have been no proof of principle experiments to demonstrate toxicity associated with different major ions is associated with consistent changes in measured TEP. In the present study, three fish species - fathead minnow (*Pimephales promelas*), channel catfish (*Ictalurus punctatus*), and bluegill sunfish (*Lepomis macrochirus*) - were exposed to different salts (KCl, NaCl, $MgCl_2$, $CaCl_2$, K_2SO_4, Na_2SO_4, $MgSO_4$, $CaSO_4$) across a range of concentrations that encompassed the fathead minnow 96-h LC50. At each concentration, the instantaneous change in TEP was measured. While there were species-specific differences in TEP response as a function of ion exposure, the change in TEP at the LC50 was relatively constant for each species. A second set of experiments demonstrated acclimation of fish for 24 h to these different salts did not elicit a change in TEP as compared to the instantaneous measurements. Additional studies are needed to characterize the effects of salt mixtures, examine effects of long-term exposures on TEP, make direct measurements of permeability coefficients, and repeat these experiments with representative invertebrates. However, results to date suggest that, at least for fish, the TEP has a strong mechanistic basis allowing for predictions of major ion toxicity.

186 Major ion toxicity to glochidia of imperiled and common freshwater mussel species

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A variety of anthropogenic activities result in major ion pollution of freshwater, posing a significant risk to aquatic communities. Freshwater mussel taxa are severely imperiled and among the most sensitive to several contaminants, including chloride. Some limited data exists on the toxicity of NaCl to mussel larvae (glochidia) but none has included imperiled species and very little is known of the toxicity of other major ions to glochidia of common or rare species, or the effects of hardness on major ion toxicity to glochidia. Therefore, the primary objectives of this work were to determine: 1) major ion acute toxicity to glochidia of common and imperiled mussel species, and 2) the relationship between water hardness and major ion toxicity to glochidia. We determined 24-hr EC50s for Fatmucket (*Lampsilis siliquoidea*) with NaCl, KCl, $MgCl_2$, $CaCl_2$, Na_2SO_4, $MgSO_4$, and $CaSO_4$ in moderately hard water. We also determined NaCl and KCl EC50s for glochidia of three rare species, Wavy-rayed lampmussel (*L. fasciola*), Fine-lined pocketbook (*Hamiota altilis*), and Alabama rainbow (*Villosa nebulosa*), to better assess the range of sensitivity among North American mussels. Effects of hardness on toxicity of NaCl were evaluated for Fatmucket, Wavy-rayed lampmussel, and Flat floater (*Anodonta suborbiculata*). Toxicity of NaCl was generally consistent with previous glochidia studies with these and other species. KCl was more toxic than all other salts by 1-2 orders of magnitude and may pose a hazard to mussel early life stages. Hardness appears to ameliorate the toxicity of chloride, similar to metals. Results of this work advance our understanding of relative risks of major ions to common and imperiled freshwater mussel species, and inform derivation of water quality standards and criteria to improve protection of aquatic species.

187 Toward establishment of a screening threshold for salts to Ceriodaphnia dubia

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Elevated total dissolved solids (TDS) can be toxic to aquatic life in freshwater systems. The composition of major ions is considered important to predict effects, and several model databases for acute toxicity prediction have been developed by individual laboratories using an array of salt combinations. However, estimates of the contribution of major ion excess to whole effluent toxicity in treated water discharges using these models or similar generalizations have proven to be inaccurate. Accordingly, we compiled and analyzed the distribution of data for major ion (Ca, Mg, Na, Cl, CO_3, SO_4) toxicity to *Ceriodaphnia dubia* from the literature and a WET testing program from a coal bed methane production operation. In-house, short-term chronic toxicity data were developed to examine the additivity of various NaCl:$NaHCO_3$ combinations, and acute study design was used to evaluate the effects of low hardness in NaCl toxicity. The results indicate that NaCl and NaHCO3 toxicity is generally additive; the ameliorative effect of Ca ions (e.g., hardness) is minor above levels which may represent calcium deficiency. Parameters that describe the overall ionic composition of the exposure waters (e.g., osmolarity, ionic strength) are similarly predictive of effects as individual ion concentrations. We find, however, variability within the broad data set prevents confident establishment of numeric TDS toxicity thresholds based on *C. dubia* response.

185 Using binary mixtures of major ion salts to assess toxicity drivers with the mayfly Neocloeon triangulifer

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Field studies have shown that mayflies (Ephemeroptera) tend to be more sensitive than other benthic macroinvertebrates to elevated levels of total dissolved solids in streams, and initial results of laboratory acute and chronic tests with the parthenogenetic mayfly *Neocloeon triangulifer* support the findings of field assessments. We previously presented data for single salts that suggested that dilution water composition influences the toxicity of major ion salts to *N. triangulifer*,as has been observed for other species. In the present study, we used binary mixtures and in one case a ternary mixture, plus D-mannitol and Na, K, and Mg gluconate salts to investigate potential drivers of toxicity in solutions with elevated major ions. D-mannitol and gluconate were used to increase osmolarity in solutions without introducing ions (or anions specifically in the case of gluconate) that can cross membranes and cause toxic effects by mechanisms other than external osmolarity. None of the single salt or mixture toxicity tests had a median lethal concentration (LC50) in terms of osmolarity that was more than 50% of the LC50 in terms of osmolarity for D-mannitol. This suggests that while osmolarity is correlated with toxicity, it does not appear to be the cause, in contrast to what has been observed for *Ceriodaphnia dubia*. We conducted acute tests with various proportions of the following binary mixtures: NaCl and Na_2SO_4; Na_2SO_4 and $MgSO_4$; NaCl and $MgCl_2$; KCl and K_2SO_4. In each case, evidence of additivity was observed, which suggested that both salts were contributing to a common mechanism of action. The additivity of the Na and Mg salts for the mayfly was in contrast to what has been documented for *C. dubia,* for which Na and Mg acted independently. This may suggest a stronger role for toxicity to the mayfly from the anions sulfate and chloride. Most published literature on major ion toxicity reports data in terms of anions (i.e., chloride or sulfate toxicity), while recent work with other species (Duluth lab) suggested that cations may be more important than anions in causing toxicity. For the mayfly, it appears that both cations and anions may cause toxicity, depending on the mixture involved and which particular ion reaches its toxic threshold activity. This abstract does not necessarily reflect USEPA policy.

189 Similarities and differences in acute response to major ions among several aquatic species: Implications for guideline development

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Adverse effects from increased concentrations of major geochemical ions (Na, K, Ca, Mg, Cl, SO_4, HCO_3) to aquatic organisms have been demonstrated or implied in many settings. However, experimental work has shown that the toxicity of ion mixtures is dependent on the specific mixture of ions present, and these vary according to both the source of the enrichment and the underlying geology of the waterbody. To date, we have conducted extensive acute toxicity testing of major ion salts (and mixtures of thereof) with a variety of aquatic species, including cladoceran (*Ceriodaphnia dubia*), fathead minnow (*Pimephales promelas*), amphipod (*Hyalella azteca*), mayfly (*Neocloeon triangulifer*), midge (*Chironomus dilutus*), oligochaete (*Lumbriculus variegatus*), and snail (*Lymnaea stagnalis*). While we expected overall sensitivity to vary across taxa, we have been surprised at the number of differences in how species respond to different ionic compositions. For example, response of some organisms to Na salts appears related to solution osmolarity, while responses of others appear more governed by Na concentration, and perhaps to SO_4 at lower Ca concentrations. For several (but not all) invertebrate species, mixtures of Na and Mg salts show independent behavior suggesting separate toxic mechanisms, but for fathead minnows and mayfly the interaction appears more additive. Toxicity of K salts is highly dependent on Na concentration in *Ceriodaphnia*, but not in fathead minnow, mayfly or amphipod. On the other hand, some responses do appear more generalizable, such as lower toxicity from Na or Mg salts at elevated (but less than directly toxic) Ca concentration. We discuss the risk assessment challenges posted by these differing characteristics in response across species. This abstract does not necessarily reflect USEPA policy.

190 Microsiemens or Milligrams: Measures of Ionic Mixtures

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In December of 2016, EPA released the *Draft Field-Based Methods for Developing Aquatic Life Criteria for Specific Conductivity* for public comment. Once final, states and authorized tribes may use these methods to derive field-based ecoregional ambient Aquatic Life Ambient Water Quality Criteria (AWQC) for specific conductivity (SC) in flowing waters. The methods provide flexible approaches for developing science-based SC criteria that reflect ecoregional or state specific factors. The concentration of a dissolved salt mixture can be measured in a number of ways including measurement of SC, total dissolved solids, freezing point depression, refractive index, density, or the sum of the concentrations of individually measured ions. For the draft method, the measure of SC was selected because SC is a measure of all ions in the mixture, the measurement technology is fast, inexpensive, and accurate, and it measures only dissolved ions. When developing water quality criteria for major ions, some stakeholders may prefer to identify the ionic constituents as a measure of exposure instead of SC. A field-based method was used to derive example chronic and acute water quality criteria for SC and two anions, a common mixture of ions (bicarbonate plus sulfate, $[HCO_3^-] + [SO_4^{2-}]$ in mg/L), that represent common mixtures in streams. These two anions are sufficient to model the ion mixture and SC ($R^2 = 0.94$). Using $[HCO_3^-] + [SO_4^{2-}]$ does not imply that the cause of benthic invertebrate decline is only due to these two anions. Measurement of either SC or of all major ions in the mixture is an acceptable exposure measure for developing AWQC for major ions. These results indicate that a criteria value derived using quantification of specific ions would be expected to be similar to the more easily measured metric of SC. These are the authors views and do not necessarily represent views or policies of USEPA.

191 Assessing the toxicity and risk of salt-impacted winter road run-off to the early life stages of freshwater mussels

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In temperate regions where road salting is used for winter road maintenance, the level of chloride in surface waters has been increasing. This is a concern for freshwater mussels, because many species, including Species at Risk have ranges limited to southern Ontario, Canada's most road-dense region. While mussel early-life stages are known to be sensitive to salt, the direct toxicity of winter road runoff to Ontario freshwater mussels had not been examined. This study examined the acute toxicity of field-collected winter road runoff (14,400 mg Cl/L) to two mussel early-life stages; glochidia (i.e. larvae; 48 h exposure, *Lampsilis fasciola*) and newly-released juveniles (Lampsilis siliquoidea). To characterize potential responses in different receiving environments, acute sensitivity was examined in waters of different hardness. The effect of chronic exposure (28 d) to runoff was also assessed using older (7-12 month old) juvenile *L. siliquoidea*. The 48-h EC50s for *L. fasciola* glochidia exposed to road run-off dilutions were similar in moderately hard (~100 mg $CaCO_3$/L; EC50 7.8% runoff, or 1177 mg Cl/L (95% Confidence Intervals 1011-1344)) and very hard synthetic water (~250 mg $CaCO_3$/L; EC50 7.0% runoff, or 1032 (739-1324) mg Cl/L). The 96-h EC50s forL. siliquoideawere 13.8% runoff (2276 (1698-2854) mg Cl/L) in moderately hard and 20% runoff (3159 (2206-4112) mg Cl/L) in very hard water. These effect concentrations correspond with the acute toxicity of chloride reported in other studies, indicating that chloride is likely the driver of toxicity in salt-impacted road-runoff rather than other compounds (metals, PAHs). The 28 day EC50 for 7-12 month old mussels was 10.6% runoff (1810 (1429-2190) mg Cl/L) in moderately hard water. Toxicity data from the current study, the literature, and concentrations of chloride in ON surface waters were used to conduct a probabilistic risk assessment of chloride to early-life stage mussels. The assessment indicated that acute toxicity of mussel early-life stages during the months for which chloride data are available (March-Nov.) is unlikely to occur, however there is a lack of data for the winter months when the majority of salt-impacted road runoff occurs. In addition, the risk assessment exercise revealed that chronic exposure to elevated chloride could pose a risk to juvenile mussels and that further investigation is warranted to ensure that these sensitive organisms are protected.

192 Predicting the Aquatic Toxicity of Mixtures of the Major Ions: Application to Additional Test Species

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Elevated concentrations of the seven major ions (Ca^{2+}, Mg^{2+}, Na^+, K^+, Cl^-, SO_4^{2-}, and HCO_3^-), usually not considered toxic, have increasingly become a concern in freshwater ecosystems. The development of water quality criteria for major ions has proven to be a challenging endeavor because toxicity of any specific ion has been found to be influenced by both the concentrations of other dissolved ions as well as by the overall mixture composition. The use of an integrative measure such as TDS or conductivity to evaluate multi-ion toxicity (MIT), on the other hand, provides an indication of the total concentration of the ions but still fails to address the mixture composition. Because of the aforementioned limitations of relating aquatic toxicity to specific ion concentrations or TDS/

conductivity, we have pursued the problem from a different perspective – by relating toxicity primarily to trans-epithelial potential (TEP). This physiological parameter is computed based on internal and external major ion concentrations and calibrated model parameter values. This approach has been successfully applied to a few relatively large acute toxicity datasets (*C. dubia*, *D. magna*, *P. promelas* and *N. triangulifer*) with widely varying ion compositions. Such datasets are limited in number and, as a result, it is necessary to also utilize smaller, less comprehensive datasets for model development. This presentation will describe an approach that has been used to incorporate these smaller data sets in the TEP model. The approach will be illustrated using results obtained with data for species not previously evaluated to date. We will also describe how the MIT approach might be extended to chronic toxicity datasets.

Fate and Effects of Metals – Metal Mixtures and Field Assessments

193 Laterite associated waters - What the lack of major ions means to trace element bioavailability and toxicity

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Numerous comparative analyses have generally concluded that tropical and temperate species are similar in sensitivity to metals. We postulate that the question of differential metal sensitivity between temperate and tropical species has not been asked within the proper framework. In additional to temperature, we suggest geology may be a major driver of organism sensitivity to metals. Specifically, laterites and bauxites are an abundant class of soils and sub-soils formed by extended weathering under tropical climatic conditions resulting in a leached profile enriched in some elements but depleted in those that are soluble under the specific conditions of formation. Specific to metals and other toxicants that disrupt ionoregulation, we hypothesize that the unusual chemistry of waters associated with laterite geology has led to the evolution of aquatic biota that use highly modified or alternative strategies to maintain ionoregulatory homeostasis and that these different strategies may impart differential metal sensitivity. The limited number of studies conducted to date, primarily on fish from the Amazon basin, support this premise. Amazon fishes show one of two strategies for ionoregulation in dilute waters: low ion uptake/low diffusive ion loss or high ion uptake/high diffusive ion loss. The latter would be predicted to be particularly sensitive to metals based on studies with temperate species, and yet several Amazon fish species that use the high uptake/diffusive loss strategy have remarkable tolerance to copper in waters with high copper bioavailability. In contrast, at least one Amazon fish is sensitive to cadmium at concentrations that are consistent with predictions based on temperate species. The majority of research into metal bioavailability and toxicity in natural waters has been undertaken on temperate or tropical species from non-lateritic geology or in laboratory waters not representative of lateritic waters. However, as exploitation of lateritic deposits becomes a more common source of base metals globally, issues related to natural aquatic ecosystem composition and sensitivity to altered metal concentrations have increased focus on the nature of freshwaters associated with these regions. This presentation examines some important characteristics of laterite-associated waters, their biodiversity, their ion chemistry and how that relates to potential trace metal bioavailability and toxicity.

194 Evaluating Bioavailability and Ecological Risks Related to Copper at Newtown Creek, New York

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Newtown Creek, a 3.8 mile-long, tidally influenced, dead-end tributary to the East River in New York City, is undergoing assessment as part of a CERCLA Remedial Investigation / Feasibility Study (RI/FS). This presentation examines the relationship of bioavailability to ecological risk of copper for benthic invertebrates, other aquatic organisms, and wildlife potentially exposed to copper at the site. The RI/FS includes analysis of total copper in sediment and tissues, in conjunction with sediment toxicity tests conducted along a gradient of copper concentrations within the study area and in nearby reference areas. Multiple lines of evidence were used to evaluate the relationship of copper bioavailability to toxicity in these tests, including analyses of dissolved copper in sediment porewater. USEPA's draft marine Biotic Ligand Model was used to interpret the effects of water quality parameters on partitioning and bioavailability of copper in porewater. Simultaneously-extracted metals (SEM) and acid-volatile sulfides (AVS) were measured under varying degrees of oxidation to evaluate the stability of particle-bound copper and other SEM metals. Mineralogical analyses including sequential extraction and X-ray diffraction were used to evaluate chemical forms of copper in bulk sediment. In addition, copper concentrations were measured in *Nereis* bioaccumulation tests, along with tissue for various free-ranging fish and crab species throughout Newtown Creek. Caged mussel studies were also conducted to evaluate copper uptake from the water column. Wildlife exposure models were used to estimate site-related copper risks for birds and mammals foraging in the study area. Collectively, these investigations provide an unusually rich dataset for examining copper bioavailability and ecological risk in an urban marine estuary.

195 Applying metal-mixtures toxicity modeling to field-collected pore waters to estimate risks to benthic fishes in a large river

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In ecological risk assessments at contaminated sediment sites, epibenthic organisms living at the sediment-water interface and in the shallow substrates are often of interest. The free flowing sections of the upper Columbia River, Washington, USA, received historical releases of smelter waste from about the 1920s to 1996 resulting in elevated metals concentrations in downstream sediments. In this section of the river, larval white sturgeon (*Acipenser transmontanus*) are thought to burrow into the substrate near the river thalweg in depths of 10m or more, and with overlying water column velocities up to 4 meters per second. Shallow pore waters were collected through pressure triggered probes and syringes attached to a 90kg torpedo-shaped weight. Potential effects to larval sturgeon were predicted using the Tox model, which is a variation of a biotic ligand model (BLM) that allows predictions of metal mixture toxicity as the sum of modeled metals accumulation on the fish gills multiplied by intrinsic potency factors that are specific to metals and endpoints. Potency factors for Cu, Cd, Zn and Pb were estimated from previous acute and chronic toxicity testing with white sturgeon. We believe that this modeling approach applied to field-collected, shallow pore waters which reflect in situ chemistry and hydrology gives a more realistic estimate of metals exposure and potential risks to benthic fishes from contaminated river sediments than would either water column sampling or pore waters centrifuged from conventional sediment sampling.

196 Hypersensitivity of a freshwater green alga to copper at low iron concentrations

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Copper is an essential trace element required for the normal functioning of cellular metabolism, but that may become toxic at high concentration. In the euryhaline green alga *Chlamydomonas reinhardtii,* growth is typically inhibited around 10^{-7} M Cu^{2+}. In this work, *Chlamydomonas reinhardtii* growth inhibition by copper ions was determined in buffered media containing a gradient in Fe (III) concentrations, another important trace element in cellular metabolism. Our objective is to understand how micronutrients, especially iron, may have a modulating effect on copper interactions with green algae. The EC50 obtained under low iron culture conditions was 4.8×10^{-12} M Cu^{2+} which is 4 to 5 orders of magnitude less than EC50s (~10^{-7} M Cu^{2+}) found in the literature. This interesting result reveals a hypersensitivity of this green alga to copper and highlights the important role of iron in copper uptake and toxicity to *C. reinhardtii.*

We hypothesise that iron and copper compete for the same transmembrane transport system (analogous to Cd/Zn interactions with membrane transporters recently documented in *C. reinhardtii*), which can reduce or exacerbate copper toxicity, depending on antecedent iron growth conditions. Key words: Copper uptake, copper toxicity, hypersensitivity, iron, competition effect, antagonistic effect, protective effect, freshwater alga.

197 The sensitivity of the test organism can influence conclusions about the additivity of metal-mixture toxicity

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Surface waters often contain mixtures of metals that are potentially toxic to aquatic life, yet mixture toxicity is currently difficult to predict due to apparent non-additive interactions among metals when based on dissolved metal concentrations. For example, the acute toxicity of binary- and some ternary-metal mixtures containing a combination of Cu and Ni to *Daphnia magna* in USEPA moderately hard water containing 3 mg/L dissolved organic carbon (DOC) is more-than-additive. We hypothesize that part of this this effect may be related to metal-metal competition for binding to DOC in the exposure water. We predict that in the presence of Ni, Cu ions are displaced from DOC and thus result in increased toxicity. To test this hypothesis, we used the geochemical-speciation software WHAMVII to predict changes in bioavailable Cu concentrations as a function of Ni and DOC concentrations. We analyzed our mixture-toxicity results in a two-way matrix: assuming either a concentration-addition (CA) or independent-action (IA) mixture-toxicity model, and using either the measured metal concentrations or the calculated metal-ion activities to predict toxicity. In binary Cu-Ni mixtures, the toxicity was always more-than-additive; however, in ternary Cd-Cu-Ni mixtures, the toxicity was less-than-additive, additive, or more-than-additive, depending on the concentrations of the varied metal and on the assumed additivity model (i.e., CA predicted the toxicity more accurately than IA at intermediate to high concentrations of the varied metal). At sub-lethal to partially-lethal concentrations, the ion activities (which account for metal-metal competition for binding to DOC and other ligands) did not reconcile the apparently more-than-additive toxicity. However, in higher concentrations that would cause 100% mortality to *D. magna*, Cu ion activities increased considerably in the presence of Ni, consistent with our prediction. Therefore, the additive/non-additive toxicity conclusion for a less-sensitive species exposed to higher Ni concentrations might differ from the conclusion for the more-sensitive *D. magna* exposed to lower Ni concentrations. These results demonstrate that conclusions about additivity of metal mixtures can depend not only on complex interactions of potentially competing toxicity-controlling geochemical mechanisms, but also on the sensitivity of the test organism to the metals.

198 They are what they eat (and breathe): Metal-mixture toxicity to aquatic insect communities

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THE PROBLEM: Better predictive models of the joint toxicity of metal mixtures to sensitive natural stream communities could be helpful in the planning, permitting, and assessment of new and ongoing mining projects. Most commonly, predictive models of the effect of metals on aquatic organisms have been developed using short-term, beaker scale, laboratory-generated toxicity data. While such approaches have the advantage of allowing efficient testing of multiple combinations, their artificial nature disconnects the results from streams in the real world. Mesocosm tests used naturally colonized substrates that were then moved to the lab for testing in experimental streams. This approach has shown success in providing controlled exposures while retaining real-world characteristics of substrate, food, and biotic interactions of aquatic insect communities. APPROACH: We previously reported tests with Zn and Cd (Metal-Mix #1) and here we show results of two sets of mesocosm tests with exposures to Zn, Cd, and Cu (Metal-Mix #2) and with Zn, Cu, and Ni (Metal-Mix 3). The testing strategy is to concurrently expose insect communities to single metals and mixtures. Single metals tests are repeated to evaluate reproducibility of the methods and year-to-year variability. Substrate colonizations were done at the same place and at the same time of the year to reduce variability. FINDINGS: Metals were strongly accumulated in sediments, periphyton, and insect (caddisfly) tissues, with the highest residues concentrations occurring in periphyton. Sensitive mayflies declined in metals treatments and effects concentrations could be predicted equally well from periphyton or water concentrations. EC50 values for some taxa differed up to a factor of 20 with smaller organisms being more sensitive, emphasizing the complexity involved with comparing sensitivities across studies and the value of repeated testing. Relative to the single metals responses, the toxicity of the mixtures was either approximately additive or less than additive when calculated as the product of individual responses (response addition). However, while toxicity was less than additive in a relative sense, absolute effects of metals to sensitive mayflies still increased in the mixture scenarios. Overall, accumulation and community metric responses in the experimental streams appear congruent with patterns seen in natural streams exposed to a suite of similar metals.

199 Toxicity of the binary mixture Cd-Zn on Lemna gibba evaluated using morphological and oxidative stress enzyme endpoints

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The presence of metals in environment represents one of the mayor concerns as they are persistent in nature, non-biodegradable and can bioaccumulate. In aquatic ecosystems may have effects on primary trophic level composed partly by vascular plants or macrophytes. As a representative species we worked with a rooted free-floating angiosperm from the family *Lemnaceae*, *Lemna gibba*. The metals evaluated were Cd and Zn, individually and as mixture. We consider important to assay these ones as they are chemically similar and naturally occurs together many times. Exposures of plants were carried out in presence or absence (controls) of Cd and Zn for 7 days. Different endpoints were determined at the end of the assays. Number of fronds, fresh weight, fronds/colonies ratio, frond area and exes length were the determined morphological endpoints. Physiological changes were evaluated as enzymatic activity of catalase, ascorbate and guaiacol peroxidases, determined at the lowest concentrations. Both metal concentrations, bringing about a 50 % inhibition of frond number (EC50) was determined. In order to compare the sensitivities of the different endpoints, NOEC and LOEC toxicity indexes were calculated. For Cd, fresh weight and fronds/colonies ratio resulted in the most sensitive, while for Zn total area was the most sensitive. Even though there was no significant difference for guaiacol peroxidase activity for Cd, it presented an increase compared to control. While the other enzymes had activity levels similar to the control. In the case of Zn, catalase and ascorbate peroxidase activities where higher than control, however neither of both presented significative differences with it. For the mixture analysis, multiple regression was used to fit the observed %frond number inhibition (%FNI) to dose expressed as dissolved metal concentration ($[M_{dis}]$). The concentration addition approach was evaluated by calculating the sum of toxic units ($\sum TU$) for each mixture test based on single EC50s. The average $\sum TU$ of all tests resulted 1,13 suggesting that this mixture presents an additive toxicity to *Lemna gibba*. An increase in the enzymatic activity was observed. Ascorbate peroxidase and guaiacol peroxidase presented the maximum increase, while catalase had a moderated activity rise.

200 Chronic effects of binary-metal mixtures of copper, cadmium, and chromium to Daphnia magna

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Most of metal mixture toxicity was studied using acute exposure. While metals are normally present in the natural environment for long periods of time, understanding choric toxicity of metal mixtures is important. The present study investigates the chronic effects of binary-metal mixtures of copper (Cu) and cadmium (Cd) and Cu and chromium (Cr) to *Daphnia magna*. A titration experimental design was used in this study, at which concentration of one metal was held constant (i.e., 1.5 µg/L Cd, 5 µg/L Cr) while concentration of the other metal was varied (i.e., 1, 3, 5, 7, 9, 11, 13µg/L Cu). A metal alone test was also conducted concurrently with the mixture test for comparison of responses of organisms to metal alone and metal mixture exposures. These concentrations were chosen based on a 14-day preliminary screening toxicity test. The definitive experiments of the present study were conducted for 21 days under the laboratory conditions of the USEPA chronic toxicity test methods. Mortality, reproduction, and body metal concentrations were measured in this study. After 21 days of exposure to Cd-Cu mixtures, additive effects on survival and reproduction of *D. magna* were found. In contrast, less than additive effects were found for Cr-Cu mixtures. Results of the present study suggest different mechanisms of toxicity of the mixtures, such as independent action of Cd and Cu and interactive effect of Cr and Cu that resulted in less-than-additive toxicity of Cr-Cu mixtures to *D. magna*. These results are useful for developing environmental water quality guidelines for metal mixtures in freshwater ecosystem.

Risk Communication Toolbox

201 Risk Communication and Anticipating Challenges

A.G. Day, ARCADIS / Environment

Risk Communication is defined as an interactive process of exchange of information and opinion among individuals, groups, and institutions. It often involves multiple messages about the nature of risk. The process engages individuals by allowing them to express concerns, opinions, or reactions to risk messages, or to legal and institutional arrangements for management. It can be an important part of site closure because without it, site closure and redevelopment can be derailed or the process lengthened due to heightened concerns. Transparency in the site closure process engages the community early in the process and allows an exchange of ideas and concerns along the way. This allows the risk communication messages to be on clean and concise. Care must be taken when meeting with community members and describing site conditions because talking about contamination can raise more concerns than alleviating them. Knowing the community and its specific concerns will help in preparation for a community meeting. Environmental risk is not always the primary concern. Each community has different goals, attitudes, history and leadership. The following examples will be discussed and the subsequent lessons learned. 1) An unused field owned by an elementary school district was proposed to be purchased by a high school district and redeveloped into a soccer field with artificial turf. During the time of vacancy, the field had become an unofficial dog park. One of the dog owners organized opposition to the redevelopment and cited concern about environmental exposures. He didn't want to lose the use of the field and just wanted to stop the project. I spoke twice at public meetings about the toxicology associated with turf fields and navigated the opposing views. 2) A major oil company's tar sand pipe line broke and tar sands entered several residences. Tar sand is viscous and the pipes need to be pressurized for flow. Nearby residents were concerned about home-grown gardening and the absorption potential. The oil company arranged for a public meeting. The residents knew that the oil company would have a representative and really wanted information on compensation for property devalue. 3) On a Caribbean island, a Canadian diplomate was concerned about orders emanating from biotreatment of fuel-affected soil adjacent to the embassy. A public meeting was held to inform interested parties on the biotreatment, monitoring and plans for soil reuse.

202 Approaches to Building Your Internal Resources

R.N. Hull, Intrinsik Corp.

As scientists, we know how to compile and analyze data, develop clear messages regarding the results of a study, prepare presentation materials that are easy to read, and make sure we know the details of the study so that we can present the material and answer questions. Even with all of this done, if you're the person who has to stand up in front of the crowd and give a presentation, you may feel anxiety and stress! How can you prepare yourself for this challenge? There are simple, evidence-based approaches to help you build your internal resources to prepare you before the meeting begins (from a few minutes before, to several days before), to assist you in responding to difficult comments or questions as they arise during the meeting, and to help you in the hours and days after a meeting has ended. To begin with, something as simple as a slow, deep breath has been shown to reduce heart rate and blood pressure. Try this before going into your meeting; it also works well before doing a SETAC presentation! Also, neuroscience shows that the brain responds to positive experiences, whether they happen in real life, or whether you imagine them. So, envision yourself giving a successful presentation. If it can work for athletes, it can work for scientists! If you find yourself waking up at 3 am worried about something that happened yesterday or may happen today or tomorrow, try a short meditation, such as focusing on your breath, or sequentially focussing on relaxing each part of your body. When you are in a meeting, don't multi-task; multi-tasking is the art of doing two or more things poorly at the same time. Be a mindful listener, paying full attention to what people are saying and doing. To minimize the potential for a rapid negative reaction, STOP: Stop, Take a breath, Observe, the Proceed with a skillful response, and be sure to THINK before you speak: ensure what you are going to say is True, Helpful, Inspiring, Necessary, and Kind. These and other short, simple, and free practices can help you in your role as a risk communicator, as a scientist, as a manager, and also in everyday life. Studies have shown that they improve focus, help manage the worry (ruminating) that can keep you up at night, help you manage stress, and make you more skillful in your reactions to difficult people and challenging situations.

203 Overview of @Risk Project: Canadian society's ability to manage risk

A. Ethier, ARCADIS

An overview will be provided of the @Risk project organized by the University of Ottawa ISSP program. The project includes a collaborative multi-disciplinary and cross-sectoral team with strong links to policy-makers and practitioners will be studying the impacts and attributes of risk perception differences over the next couple of years. The goal is to compare and evaluate risk management frameworks and broader policy and regulatory decisions in energy, health and genomics policy sectors in an attempt to incorporate public values and concerns into evidence-based decision making.

204 From Risk Communication to Stakeholder-driven Hypothesis Testing

I. Linkov, B.D. Trump, US Army Engineer Research and Development Center; M. Wood, US Army Engineer Research and Development Center / Environmental Laboratory; J. Palma Oliveira, University of Lisbon

Where governments, project planners, and commercial developers seek to develop new infrastructure, industrial projects, and various other land and resource-intensive tasks, veto power shared by various local stakeholders can complicate or halt progress. Risk communication has been used as an attempt to address stakeholder concerns in these contexts, but has demonstrated shortcomings. Specifically, coordination failures between project planners and stakeholders can arise where local publics resist or impede project development due to perceptions of potential harm, such as with the potential for chemical pollution and contamination of local water and soil. To overcome such coordination failures, we demonstrate how a two-step process can directly address public mistrust of project planners and public perceptions of limited decision making authority. This approach is examined via two separate empirical field experiments in USA, Portugal and Tunisia, where coordination failures and public resistance threatened to derail emerging industrial projects. In both applications, an intervention is undertaken to address initial public resistance to such projects, where specifically public stakeholders and project sponsors collectively engaged in a hypothesis testing process to identify and assess human and environmental health risks associated with proposed industrial facilities. Specific efforts were taken to examine exposure of local communities to specific contaminants. These field experiments indicate that a rigorous attempt to address public mistrust and perceptions of power imbalances and changing the pay-off structure of the given dilemma may help overcome such anticommons problems in specific cases, and may potentially generate enthusiasm and support for such projects by local publics moving forward.

205 Employees, the Forgotten Audience: Risk Communication Tools to Drive Internal Engagement

S. Perry, B. Butterly, ARCADIS US Inc / Technical Communications

Risk assessment results and risk management solutions often affect employees within a facility or company, yet how well do we engage employees to help them truly understand the data, drivers, and potential need to change behaviors and procedures to reduce a risk or underlying hazard? Answer: not very well; experience shows that employees are too often the forgotten audience. As risk communicators, we need to take a fresh look at our assumptions and toolbox to make sure we address internal/employee needs just as seriously and completely as the external demands of the public, media, and intervenors. One key difference we'll explain is the role of empathy, which is a necessary ingredient in all effective risk communication. How and how much empathy is used depends on the audience, objective, and other technical and situational factors. Successes and lessons learned from several industries will be shared. For example, we'll show how traditional external risk communication tools can be successfully adapted for internal/employee use, including how risk assessment results, words, and visuals must be modified to become more personal and practical for assimilation by an affected employee. We'll also share insights on other critical tools, including how to select and prepare an internal spokesperson faced with managing challenges quite different than the typical external spokesperson's role. Finally, some cases benefit from a dedicated hotline or employee Q&A tool that helps business leaders and risk communicators identify leading indicators and emerging issues well before they escalate into the misperception and misinformation that can undermine our risk management objectives.

206 Ecological risk communication what it is not and how we may be able to do it

M.D. Sprenger, USEPA / OSRTI TIFSD ERT

Risk communication has always presented a challenge to risk assessors. Ecological risk assessment guidance refers to the need for ecological risk communication, however, to date there is no formal guidance on how ecological risk communication should or can be effectively accomplished. Typically, what is presented as ecological risk communication is actually information transfer and is often only useful to other ecological risk assessors or individuals within related technical fields. Attempts at communication between the ecological risk assessors and others, such as decision makers or the public, have been a topic of concern throughout the development of the ecological risk assessment process. Adding to the dilemma faced by ecological risk assessors is a changing landscape of communication in general, from hard copy guidance documents and articles to electronic and visual formats. However, embracing these new formats may present an opportunity for ecological risk communication to finally advance.

207 Strengthening Your Communication Skills: Exercises to Build Rapport

H.L. Govenor, Virginia Tech / Biological Systems Engineering; C. Kroehler, Virginia Tech / Center for Communicating Science

Developing strong communication skills takes thoughtful practice. While it may seem obvious that effective communication requires the use of a common language and active listening, these goals may be more easily said than achieved. In this session we will share fun and thought-provoking exercises drawn from science communication training coursework. Session participants will engage in exercises borrowed from theatre practices and designed to improve listening skills. We will also demonstrate the dangers of jargon, describe strategies for avoiding words with multiple meanings, and provide links to resources that can be used on a daily basis to assist scientists and professionals in communicating their work to each other and to public stakeholders. http://communicatingscience.isce.vt.edu/resources.html

208 Panel Discussion of Effective Communication Tools for Human and Ecological Risk Communication - A Toolbox for Environmental Management

B. Mullhearn, Ensafe Inc; S. Sager, Arcadis JSA; J. Clarkson, Louisiana Department of Natural Resources; N. Sengupta, Journal of Visualized Experiments (JoVE)

Risk communication is the interaction between environmental risk assessment scientists, managers, policy makers and the interested public. With information readily available from the internet, mass media, and "24/7" journalism, communicators need to be cautious and thorough when interpreting and communicating information to stakeholders. Many environmental scientists, managers, and policy makers are uncomfortable in the role of risk communicator, and they have often overlooked the importance of being able to communicate effectively with the interested public. Therefore, risk communication remains one of the most underused tools for environmental management. This panel discussion will engage the audience and all of the speakers for the session in a two-way conversation designed to highlight effective risk communication strategies as tools for use by science and risk communicators. The co-chairs for the session will moderate the discussion. Each presenter will provide a single summary slide to the moderators, who will review and collate the information. The combined summary will be used as a starting point for discussion and for drawing questions and comments from the audience. The moderators will also create a list of questions and comments for the panel, to ensure a robust and lively discussion is presented. If needed, the panel can respond to any questions that were not addressed during the individual presentations due to time constraints. Communication is an integral and critical element in any risk management process. Failure to effectively communicate can result in a breakdown of this process. Ideally, this panel discussion has the potential to bring together experts from government, industry, and academia to review shared experiences about risk communication for human and ecological health. If possible, this panel discussion could culminate in a "communication toolbox" that can be used by environmental scientists, managers, policy makers, and others in a variety of environmental situations.

Fate and Effects of Chemicals from Diffuse Sources and Stormwater

210 Development and Application of a Copper Roof Runoff Assessment Tool

C.J. Fanelli, R.F. Carbonaro, Mutch Associates, LLC; J. Moore, Towson University / Geosciences; R.E. Casey, Towson University / Chemistry; K.J. Rader, Mutch Associates, LLC

Copper (Cu) has been used in exterior architectural applications for centuries because of its aesthetic appeal, durability, and low maintenance requirements. Within the last 20 years, elevated copper levels in copper roof stormwater runoff combined with copper sensitivity of some aquatic species (e.g. salmon) have resulted in increased scrutiny and regulation of these copper applications and others representing diffuse copper sources to the environment. The work presented here focuses on copper release from copper roofing materials in stormwater runoff, but the concepts are applicable to many diffuse sources of copper and other metals. Observations of (1) elevated copper levels in stormwater runoff leaving copper roofs, and (2) copper's ability to cause a toxic response in sensitive organisms does not present a complete picture of the overall risk associated with architectural copper. Consider the following three "A's": (1) *Amount* or how much copper is released and at what concentration, (2) *Attenuation* or how much copper is removed from stormwater between the roof and the point at which aquatic organisms encounter the runoff, and (3) *Availability* or how much of the copper in runoff exists in a form that can cause toxicity. All three play an important role in determining the potential impact of copper in runoff. The goal of this work was to use copper runoff rate and attenuation data from two-year study conducted at Towson University along with local stream hydrology and chemistry data to illustrate application of a runoff assessment tool. This tool considers the three "A's" listed above and provide a means of linking copper roof installations with potential effect in receiving waters in a quantitative manner. Analysis with a screening level tool indicates that dilution and water chemistry play a large role in determining the magnitude of the ecological impact in the receiving water. For high flow systems with protective water chemistry, a large area of copper roof is possible before adverse effects are predicted to occur.

211 Seasonal Toxicity Observed with Amphipods (Eohaustorius estuarius) at Paleta Creek, San Diego Bay, USA

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Paleta Creek (San Diego Bay, California, USA) was identified by the California State Water Board as a high priority candidate toxic hot spot due to repeated amphipod sediment toxicity observations and the presence of multiple degraded benthic communities (SCCWRP 2011 and SPAWAR 2005). Standard 10-d amphipod (*Eohaustorius estuarius*) survival bioassays were performed *ex-situ* using intact sediment cores collected from the site on five occasions between 2015-2017 (July 2015, October 2015, February 2016, September 2016, and March 2017). In addition, a subset of October 2015 sediment cores were manipulated with a top layer of deposited particles (collected from 4-month deployed sediment traps placed at each station during the 2015-2016 wet season) as an additional measure of contributions of storm water particulates on contaminant exposure and bioavailability. Dry season events (July 2015 and September 2016) cores were largely non-toxic to amphipods, but wet season events routinely showed a spatial gradient of increased mortality with increasing proximity to the mouth of Paleta Creek, where up to 100% mortality occurred. In addition, mortality was greatest when the deposited particulates (either associated with stormwater or resuspension events) from sediment traps were added to relatively non-toxic dry weather cores. These results suggest these deposited particulates are contributing to the recontamination of these sediments, and that the observed toxicity is relatively ephemeral. We quantified metal (Cd, Cu, Hg, Ni, Pb, Zn) and organic contaminant (PAH, PCB, and pesticide) concentrations to assess possible causes of toxicity. Thus far, pyrethroid pesticides are the most highly correlated with amphipod toxicity. Summing the individual pyrethroid constituents using a toxic unit approach suggests that the observed toxicity to *E. estuarius* could be associated with pyrethroids.

212 Assessing sediment recontamination due to stormwater

D.D. Reible, Texas Tech University / Civil and Environmental Engineering; B. Rao, D. Athanasiou, Texas Tech University / Civil, Environmental, and Construction Engineering; I. Drygiannaki, M. Bejar, Texas Tech University; M. Rakowska, Texas Tech University / Civil and Environmental Engineering; M. Otto, B. Steets, R. Pitt, Geosyntec Consultants; C. Marienne, SPAWAR Systems Center Pacific; G.H. Rosen, SPAWAR Systems Center / Energy and Environmental Sustainability; B. Chadwick, Space and Naval Warfare Systems Center Pacific / Environmental Sciences; G. Burton, University of Michigan / School of Natural Resources & Environment

Cleanup at contaminated sediment sites has often been initiated before land-based sources have been fully identified and controlled. Our study objective was to develop, apply and test techniques to assess the magnitude and characteristics of solid-associated contaminants to sediments as a result of episodic storm events. Paleta Creek at Naval Base San Diego (NBSD) was selected for monitoring and modeling of stormwater and receiving system responses . Composite samples were analyzed for a variety of metals and organic contaminants as a function of particle size. Receiving waters were monitored for flow trajectory, sediment deposition and contaminant water, porewater and sediment concentrations. Sediments collected at outfalls, deposition traps and sediment deposits were also characterized. *Macoma nasuta* was used to determine bioaccumulation through the deployment of a Sediment Ecotoxicity Assessment Ring (SEA Ring) *in-situ* as well as, passive sampling(28 d) and *ex-situ* standardized laboratory testing. Flow and concentration data from specific events were used to calibrate a stormwater model, WinSLAMM, to allow predictions beyond the period of sampling, comparison with regulatory allowed loads and concentrations, and for future implementation scenario evaluation. The combination of physical, chemical and biological data were used to identify the most efficient and effective stormwater assessment approaches to evaluate risk of sediment recontamination, which is a function of mass loading rates of, and pollutant concentrations (relative to site specific sediment cleanup criteria and ecological toxicity thresholds) on, solids in the particle size fractions that settle in the near field. Stormwater size segregated loads combined with depositional sediment, as assessed by thin surficial sediment layers and sediment traps coupled with passive sampling and bioassays, were identified as the most useful information for assessing recontamination. Contaminants – particularly PAHs and PCBs – were often found associated with coarse particles that contributed to the mass burden in near-field sediments, but did not lead to an associated increase in porewater concentrations or bioaccumulation. Results suggest that analysis of bulk suspended sediment pollutant concentrations may underestimate concentrations on near-field deposited sediment but overestimate pollutant mass loading, and that bioavailability may be limited.

213 Nitrate acute toxicity and effects on metamorphosis in common and imperiled freshwater mussels

A. Moore, University of Georgia / Interdisciplinary Toxicology Program; C. Barnhart, Missouri State University / Biology; P. Johnson, Alabama Department of Conservation and Natural Resources / Alabama Aquatic Biodiversity Center; R.B. Bringolf, University of Georgia / Interdisciplinary Toxicology Program

Nutrient pollution, specifically nitrate, has become one of the most prevalent causes of water quality degradation globally, with increasing anthropogenic input from suburban and agricultural runoff, municipal wastewater, and industrial waste. Water quality and contaminants have been identified as major challenges for freshwater mussel populations, many of which are highly imperiled throughout North America and the world. The potential effects of nitrate to freshwater mussels are largely unknown, particularly during the parasitic stage of the complex freshwater mussel lifecycle. Therefore, we investigated the effects of nitrate on freshwater mussel larvae (glochidia) viability, attachment success on host fish, and metamorphosis success to the juvenile stage. In the first experiment, we exposed *Lampsilis siliquoidea* and *L. fasciola* glochidia to environmentally relevant nitrate concentrations (0, 50, or 250 mg NO3/L) for 24 hours before inoculation on their primary host, largemouth bass (*Micropterus salmoides*). In a separate experiment, we exposed largemouth bass to the same nitrate treatments for 28 days prior to inoculating with unexposed *L. siliquoidea* glochidia to determine if the effects of these compounds are mediated through the host fish. In a third experiment, we exposed brooding female *L. siliquoidea* to a range of nitrate concentrations (0, 50, 100, 150, 200, 250 mg NO3/L) for 25 days and monitored the same suite of endpoints to determine the effects of nitrate when glochidia are exposed during brooding in the marsupial gills. These results can be incorporated into a stage-based population model for prediction of the effects of nitrate pollution on freshwater mussel populations. Additionally, we will present 24-hr EC50s for glochidia viability for 5 species of freshwater mussels, including common and imperiled species. This will allow us to compare the sensitivity of more common species to that of imperiled species. Results of these studies are important for improving characterization of the hazards of nitrate pollution to aquatic life and this work will better define the role of water quality in assessing habitat suitability for mussel conservation efforts.

214 Organic Contaminants in Urban Stormwater: Occurrence, Effects, -Omics, and Iron-Amended Sand Filter Performance

D. Fairbairn, Minnesota Pollution Control Agency; S.M. Elliot, USGS; B.M. Westerhoff, St. Cloud State University; M.L. Ferrey, Minnesota Pollution Control Agency / Environmental Outcomes; R.L. Kiesling, USGS; H.L. Schoenfuss, St. Cloud State University / Aquatic Toxicology Laboratory; D. Martinovic-Weigelt, University of St. Thomas

To date, there are few studies of trace organic contaminants (OCs) in stormwater or stormwater management infrastructure. In contrast to sanitary wastewater, not all stormwater is required to be treated prior to discharge. Urban runoff may thus transport a broad array of OCs associated with myriad human processes and materials to surface water. In the present study, we collected samples from three large stormwater "integrator" sites and from the (untreated) inlets and (treated) outlets of three iron-amended sand filters during snowmelt and three seasonal rain events in Minneapolis-St. Paul. We analyzed approximately 400 OCs including veterinary and human pharmaceuticals; urban, agricultural, and mixed-use pesticides; and other components of urban, industrial, manufacturing, or household applications. More than 120 OCs were detected. Methyl-1H-benzotriazole, cotinine, atrazine, and 2,4-D were detected in all samples. Individual OC concentrations ranged from 0.007 to 11.6 µg/L among all sites. Total OC detections and concentrations were reduced in treated versus untreated samples. Of 48 OCs detected in ≥25% of samples, concentrations of 15 were significantly reduced in treated versus untreated samples, and 35 demonstrated seasonality. In addition, we exposed model aquatic organisms to stormwater samples to assess phenotypic and transcriptomic changes. Here, we report the identified chemical and toxicological patterns in stormwater, with a focus on method alignment and the mitigation potential of these full-scale stormwater treatment installations. These results provide a step in understanding environmental contamination, ecotoxicity, and potential mitigation strategies to protect health of aquatic organisms.

215 Biological Effects of Septic Seepage on Resident Fish Species in Minnesota Lakes

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The potential for on-site wastewater treatment systems ("septic systems") to represent non-point source of contaminants into lakes is a growing concern. Many lakes are down-gradient from septic systems, and their seepage may infiltrate shallow groundwater and enters the shallows of adjacent lakes though hydrological processes. Five study lakes were established, each with septic-influenced and reference sites. Water sampling throughout the early spring and summer established the presence and absence of contaminants at each site. Adult male sunfish were collected off of their spawning beds between May and July to assess contaminant related endpoints included histopathology of gonad, liver, and gill tissues, and analysis of vitellogenin, estradiol, and 11-keto testosterone in plasma samples. To explore the effects of contaminants on larval larval fathead minnows , a 21-day static renewal exposure was completed using pore water collected from all study sites . Following exposure, larvae underwent behavioral testing for predator avoidance performance and feeding efficiency. Elevated vitellogenin levels ($p = 0.01$, t-test) and a reduction in liver size ($p = 0.01$, t-test) were observed in resident males from septic-influenced sites. Larval fish exposed to septic-influenced pore water showed increased growth ($p =0.002$, ANOVA) and a reduction in survival ($p< 0.001$, ANOVA). This study provides evidence for reduced health and feminization of resident male sunfish, and reduction in survival of juvenile fathead minnows exposed to waters from septic-influenced areas of these lakes. Our results provide a direct link between septic system seepage and biological effects. The assessment of biological endpoints in sunfish and laboratory exposed fathead minnows provides a rich data matrix to support that contaminants from septic seepage cause adverse health effects in resident fish populations in northern lakes.

216 Fate, bioavailability and treatability of polycyclic aromatic hydrocarbons (PAHs) in stormwater pond sediments throughout Minnesota

C. Huang, University of Minnesota / Civil, Environmental, and Geo-Engineering; J. Trautman, University of Minnesota / School of Public Health; M.F. Simcik, University of Minnesota; R.M. Hozalski, University of Minnesota / Civil, Environmental, and Geo-Engineering

Presentation Type: Platform Preferred Track: Integrated Environmental Assessment and Management Session: Assessment and Management of PAHs in Urban Waterways Abstract Title: Fate, Session: Assessment and Management of PAHs in Urban Waterways Abstract Title: Fate, bioavailability and treatability of polycyclic aromatic hydrocarbons (PAHs) in stormwater pond sediments throughout Minnesota Authors: C.K. Huang, J. Trautman, M.F. Simcik, R.M. Hozalski, University of Minnesota Abstract: Polycyclic aromatic hydrocarbons (PAHs) have been detected in the sediments of several urban stormwater ponds in Minnesota, in some cases at concentrations that exceed the Minnesota Pollution Control Agency's recommended limits for unrestricted use. For such ponds, the owner would be subjected to prohibitively high sediment disposal costs. The overall extent of the contaminated pond sediment problem is not known, especially outside of the Twin Cities metropolitan area. In this investigation, twenty stormwater ponds in the metro (5 in Minneapolis-St. Paul) and outstate (5 each in Duluth, St. Cloud, and Rochester) areas were sampled to quantify PAHs in pond sediments and to identify major sources of PAH contamination to stormwater detention ponds. The concentrations of 34 parent and alkylated PAHs were determined along with measurement of sediment moisture content (MC), total organic carbon (TOC), and black carbon (BC). The bioavailability of PAHs in the sediments is being determined using a solid phase microextraction (SPME) method, which will aid in assessing potential sediment toxicity and treatability of the PAH-contaminated sediments. To date, we have determined that total PAH_{34} concentrations of the metro area pond in the range of 1.58 – 16.04 mg/kg dry wt., with an average of 9.11 mg/kg dry wt..Four high molecular weight PAHs (HPAHs) (i.e., fluoranthene, pyrene, benzo[a] pyrene, and chrysene) accounted for about 50% of the total PAH_{34} concentration, which indicates that the PAHs with four or more rings have lower degradation rates, as compare to two or three rings PAHs. This study is crucial for the development of guidelines for the remediation of PAHs in stormwater pond sediment, which are needed to lower the contaminated sediment disposal and pond maintenance costs.

Soil Contaminants – Fate, Bioavailability, Environmental Toxicology – Part 2: Application in Human Health Risk Assessment

217 Evaluating Bioavailability of Chemicals from Contaminated Soil for use in Human Health Risk Assessment: Guidance from ITRC

Y. Lowney, Alloy, LLC / Health Sciences; K. Durant, Delaware Department of Natural Resources & Environmental Control; C. Sorrentino, California Department of Toxic Substances Control

The Interstate Technology and Regulatory Council (ITRC) has convened a team of leaders in soil bioavailability testing to develop consensus-based regulatory and technical guidance on soil bioavailability for use in human health risk assessment. The goal of the project is to help regulators and practitioners select and properly use site-specific bioavailability testing to reduce uncertainty in the risk assessment process, understand the pros and cons of different in vivo and in vitro methods, and determine which method is most appropriate for site conditions. The product of this effort will be a consensus-based, web-based document that will represent the shared knowledge of representatives from state and federal regulatory agencies, the private sector, academia, and tribal and public stakeholders. It also will provide information on available bioavailability and bioaccessibility tests, including what the user should consider to make informed decisions for a specific site. An Internet-based training course will also be developed. The ITRC guidancewillinclude case studies that will show how bioavailability of lead, arsenic and PAHs have been evaluated at sites. It will discuss the challenges, how these challenges were overcome, and the lessons learned. In vivo methods can provide us with insights into site-specific bioavailability; however, the high cost and duration of these in vivo studies severely limit their applicability to a small number of large sites where there are considerable resources available and a long timeline. In the past few years, various groups have developed in vitro methods to measure bioaccessibility as a surrogate for bioavailability. These in vitro methods are available for arsenic (As) and lead (Pb) and their relatively low cost and turnaround time allow for the inclusion of site-specific bioavailability considerations for lower- budget sites. However, not all in vitro methods will necessarily work well with all types of soils and chemical forms of the contaminant. Accordingly, the decision on the suitability of any one method to determine bioaccessibility should take into consideration site-specific conditions, including which agency has jurisdiction. A one-size-fits-all approach is not possible for evaluating the bioavailability of contaminants in soil because of the inherent complexities. The new guidance from ITRC will provide regulators, stakeholders, and practitioners with the tools they need to make informed decisions.

218 The impact of lead bioavaiability on Lead Exposure Assessments of contaminated sites in Jamaica

T. Ramikie, University of the West Indies / Chemistry

The impact of soil lead bioavailability on exposure assessments is well documented in developed countries. However, in tropical developing countries such as Jamaica, few studies address its importance. An assessment of lead bioavailability in Jamaican soils is necessary as, over the past 40 years, cases of lead poisoning have consistently occurred. Exposure studies have indicated that the main pathway to human uptake is soil ingestion and hence a determination of lead bioavailability in Jamaican soils can significantly improve community health risk assessments. Given this need, this study seeks to assess the lead bioavailability of in soils from sites previously found have high lead contamination.). These sites are: Mona Commons and Kintyre, both in the Parish of St Andrew, and Fraser's Content in Parish St Catherine. The chosen sites have different soil characteristics, are now densely populated and have a history of childhood lead poisoning. Investigations were conducted using the Relative Bioavailability Leaching Procedure (RBLP) which is an in-vitro technique assessed and approved by the USEPA for specific site assessments of lead contamination. Three runs were conducted using representative samples of particle size < 250µm from each site. The results indicate relative bioavailability (RBA) of Fraser's Content, Mona Commons, and Kintyre are 61, 58% and 44%, respectively. The results are within the range reported in other countries (USA, Australia) with similar sources of contamination. It was concluded that: 1) the RBLP methodology can be used to assess lead bioavailability in Jamaican soils and 2) the three lead contaminated sites exhibited different degrees of lead bioavailability, 3) The degree of lead contamination positively correlate with the number of lead poisoning cases reported within these communities. Thus, estimating lead bioavailability using the RBLP methodology is an effective way of determining lead bioavailability in tropical loam and clay soils and impacts environmental exposure assessments.

219 In vivo Validation of the Mehlich 3 as a Soil Pb Bioaccessibility Assay

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Lead (Pb) is an insidious and ubiquitous soil contaminant in urban areas. Blood Pb is associated with impaired cognitive function including increased incidence of ADHD, reduced test scores, and behavioral issues even at very low blood Pb concentrations (e.g. < 5 µg/dL). Accurate estimation of soil Pb health risk requires knowledge of soil Pb bioavailability i.e. the fraction of total soil Pb that can be absorbed by the body. Pb absorption by swine is the animal model considered most representative of Pb absorption by young children but the extreme resource and time requirements for swine analyses make them unavailable to the public. Identifying a simple, common, and inexpensive soil test that is well correlated with swine feeding trials could dramatically reduce economic and logistical barriers that prohibit soil accurate Pb bioavailability estimation. The Mehlich-3 is a common, inexpensive soil nutrient test hypothesized to be well correlated with Pb bioavailability because of its correlation with the in vitro Pb bioaccessibility test the Relative Bioavailability Leaching Procedure at pH 2.5. However, in vivo validation for the Mehlich-3 is lacking. This study seeks to improve public access to Pb bioaccessibility testing by evaluating the ability of the Mehlich-3 to estimate Pb bioavailability as determined through swine feeding trials. Four uncontaminated soils from Queensland and South Australia, Australia were spiked with Pb acetate to a concentration of 1500 mg kg^{-1}, then aged until changes in soil Pb bioavailability reached a steady state. Spiked soils were sieved to < 250 µm then orally administered via soil slurries to pigs under fasting conditions. Soil relative bioavailability was determined by measuring blood Pb levels of swine fed these soils. The final stage of this project consists of extracting the four soils following the Mehlich-3 procedure. Results relating Mehlich-3 extractable Pb to bioavailable Pb are forthcoming.

220 Improving in vitro predictions of polycyclic aromatic hydrocarbon bioavailability from ingested soil

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Predicting mammalian bioavailability of PAH mixtures from in vitro bioaccessibility results has proven to be an elusive goal. In an attempt to improve in vitro predictions of PAH soil bioavailability we investigated how energetic input influences PAH bioaccessibility by using a high and low energetic shaking method. Co-inertia analysis (COIA), and Structural Equation Modelling (SEM) were also used to examine PAH-PAH interactions during ingestion. PAH bioaccessibility was determined from 14 historically contaminated soils using the fed organic estimation of the human simulation test (FOREhST) with inclusion of a silicone rod as a sorption sink and compared to bioavailability estimates from the juvenile swine model. Shaking method significantly affected PAH bioaccessibility in the FOREhST model, with PAH desorption from the high energy FOREhST almost an order of magnitude greater compared to the low energy FOREhST. PAH-PAH interactions significantly influenced PAH bioavailability and when these interactions were used in a linear model, the model predicted benzo(a)anthracene bioavailability with an slope of 1 and r^2 of 0.66 and for benzo(a)pyrene bioavailability has a slope of 1 and r^2 of 0.65. Lastly, to confirm the effects as determined by COIA and SEM, we spiked low levels of benzo(a)anthracene into historically contaminated soils, and observed a significant increase in benzo(a)pyrene bioaccessibility. By accounting for PAH interactions, and reducing the energetics of in vitro extractions, we were able to use bioaccessibility to predict bioavailability across 14 historically contaminated soils. Our work suggests that future work on PAH bioavailability and bioaccessibility should focus on the dynamics of how the matrix of PAHs present in the soil interact with mammalian systems. Such interactions should not only include the chemical interactions discussed here but also the interactions of PAH mixtures with mammalian uptake systems.

221 Effect of source materials on PAH bioaccessibility assessed by physiologically based extraction tests

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Incidental soil ingestion by humans is considered the primary pathway for systemic exposure to PAHs in soils. In this study, a simulated physiologically-based extraction test (PBET) was used to assess the PAH bioaccessibility from a library of soils previously constructed with different typical PAH source materials (fuel oil, soot and coal tar based skeet), soil organic matter, and minerals to achieve different PAH concentrations. Source material had a strong impact on PAH bioaccessibility: soils spiked with soot and skeet generally exhibited the lowest bioaccessibility, followed by fuel oil and solvent spiked soils. Among all soil compositions, the presence of 2% charcoal had the greatest effect on reducing PAH bioaccessibility, with the biggest reduction (of approximately 80%) observed for soils spiked with solvent and fuel oil. Across all soils constructed, soil partitioning coefficients (K_D) were observed to be negatively correlated ($p< 0.01$) with PAH bioaccessibility, with R^2 ranging from 0.65 to 0.74. We demonstrate that a partitioning based model that accounts for the sorption capacity of the simulated gut fluid is able to reasonably predict PBET extraction for most soils across 5 orders of magnitude in PAH mass extracted. Further investigation may be necessary to validate these results using in-vivo studies.

222 In vitro extraction tests for prediction of in vivo oral relative bioavailability of polycyclic aromatic hydrocarbons (PAHs)

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In vivo methods to directly measure the oral bioavailability of PAHs in different matrix types are usually required by regulatory agencies to support a site-specific relative bioavailability (RBA) of contaminants for use in human health risk assessment. However, the cost and time commitment associated with in vivo studies make them impractical for most sites. The development of simple and reliable in vitro methods to predict oral bioavailability of PAHs from soil is therefore a high priority. We previously measured the relative oral bioavailability (RBA) of radiolabeled benzo(a)pyrene (BaP) from a variety of constructed, weathered soil in rats (Roberts et al., Environ. Sci. Technol.50:11274-11281, 2016) in rats. That study found substantial differences in BaP RBA depending upon the source material and, to a lesser extent, on soil characteristics. The objective of the study presented here was to determine the ability of simple in vitro extraction tests to predict the RBA of BaP in these soils. Three different in vitro extraction methods were evaluated — simplified physiologically based extraction technique (PBET), simple solvent extraction using n-butanol, and EPA's modified 3550C — and correlation coefficients between in vivo and in vitro RBA estimates were calculated. Results with a simplified PBET were relatively reproducible for a given soil, with an in vitro to in vivo correlation (IVIVC) correlation (R^2) = 0.57. A simple solvent extraction of soils using n-butanol had a higher IVIVC, with an R^2 = 0.74. Extraction with EPA Method 3550C was not a good predictor of relative oral bioavailability as measured in rats (R^2 = 0.43). These results suggest that in vitro extraction methods may provide meaningful predictions of oral RBA for BaP and other PAHs. Supported in part by a grant from SERDP.

223 Calibration of Soils for Skeet-target Contamination: A Case Study from a Navy Installation

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The presence of fragments from clay pigeons (skeet) in the soils of Navy target-practice fields presents a potential health hazard due to the polyaromatic hydrocarbons (PAH) that may still be present in the soil. The source of the PAH is the tar pitch and other aromatics used to bind the minerals together in the original manufacture of the skeet targets. A great need exists to determine the actual risk posed by these materials by estimating the residual amount of skeet and PAH in the soil and its potential for entering the human food chain. The purpose of this study was to measure the level of skeet contamination in soils, using one Naval installation in Florida as a case study. Five soil samples were collected from or near the target field and dry sieved into three particle-size fractions (>2000 µm esd (equivalent spherical diameter), < 2000 to >500 µm esd, and < 500 µm esd). Each fraction was then analyzed for mineralogy, total inorganic carbon, and organic carbon using powder X-ray diffraction (XRD), thermal analysis, carbon analysis, Fourier-transform infrared (FTIR) spectroscopy, and near-infrared spectroscopy (NIR). A nearby uncontaminated soil was used as the background for baseline information. Each of the methods was calibrated for skeet content using the pure phases of three commonly used skeet materials, then the skeet content in each of the different particle-size fractions of each soil was estimated. Results indicated that some samples had no skeet, whereas others had a modest level. Interestingly, only the >2000 µm esd size fraction contained any skeet, and the two lesser size fractions were devoid of skeet regardless of the sample. This indicates that, over the years, the skeet fragments have not weathered into the smallest or colloidal size fractions, suggesting that colloidal transport of the residual skeet and its accompanying PAH would be highly unlikely. Likewise, airborne transport would also be unlikely because of the resistance of large particles to being swept up by wind. This result further suggests that water transport would also be slow because the larger particles would not readily suspend in water and thus would settle rapidly even if caught up in flowing water.

224 Application of Chemical Availability Measurements for Evaluating PAH Risks in Soils Impacted with Clay Targets

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A major uncertainty of many site-specific human health and mammalian risk assessments is the digestive bioavailability of Polycyclic Aromatic Hydrocarbons (PAHs) consumed via the incidental ingestion of contaminated soils. Bioavailability can be quantitatively considered in site-specific risk assessments using a Relative Bioavailability (RBA) value, which is often conservatively assumed to be 100% unless lower values can be provided from the results of a laboratory experiment with rodents fed small amounts of site soils. We have evaluated an approach to measure the availability of PAH in soils using two chemical availability tools that are much more time- and cost-effective than animal tests: 1) Physiologically Based Extraction Tests (PBET) that extract PAHs from soil using a simulated digestive matrix (in vitro bioavailability approach); and 2) determination of freely-dissolved PAHs in soil via a commercially-available polyethylene passive sampling devices. Using an empirical model relating the chemical availability measurements and animal bioavailability data, the human health risk of benzo(a)pyrene (BaP, the most potent PAH in terms of carcinogenicity) can be predicted for soils using PBET and passive sampler data in a manner that accounts for soil-specific availability. To demonstrate this approach, 6 soils impacted with clay target fragments (a source of PAHs with limited bioavailability) and 2 reference soils ranging from 0.03 to 2600 mg BaP/kg soil, dry weight were evaluated using the PBET and passive sampling approach. Based on the default RBA of 100%, 5 of the 8 soils exceeded a residential risk threshold of 1×10^{-4}. However, the chemical availability approaches indicated BaP risk threshold exceedances for only 1 (based on PBET) or 3 (based on passive sampling) of the 5 soils due to the lower RBA estimates (0.2% to 7% for PBET and 3% to 60% for passive samplers, respectively). Application of this approach at sites impacted with clay targets (or other sources of PAHs with limited bioavailability) would enable PAH risk assessment to account for site-specific availability without the reliance on animal testing.

Aquatic Toxicology and Ecology – Part 1

225 Comparative analysis of exposure and physiological response to PAHs and PCBs in three coastal sharks

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Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) are ubiquitous within coastal marine environments. These lipophilic compounds differ in bioaccumulation potential since many organisms can readily metabolize PAHs and coplanar PCBs, but take longer to biotransform non-coplanar PCBs. Sharks are useful bioindicators of coastal pollution since many species are long-lived, feed at high trophic positions, and occupy a variety of aquatic habitats. Exposure of sharks to PAHs and PCBs has often focused on body burdens or biomarkers exclusively, rather than through integration of both methods. This study uses an integrative approach by correlating PAH and PCB body burdens with that of cytochrome P450 1A1 (CYP1A1) and glutathione-S-transferase (GST) activity. Bull (*Carcharhinus leucas*), blacktip (*Carcharhinus limbatus*), and bonnethead (*Sphyrna tiburo*) sharks were sampled from highly-impacted Galveston Bay, TX to compare the effects of feeding ecology and life history. Ontogenetic differences were also analyzed to determine whether certain age-classes may be more vulnerable to bioaccumulation of these contaminants. Inter-specific differences in body burdens are present, as well as differences in 7-ethoxyresorufin-O-deethylase (EROD) and GST activity. These results demonstrate differences in exposure and physiological response to PAHs and PCBs based upon multiple factors. Positive and negative correlations were quantified for body burdens of individual PAHs and PCB congeners with that of EROD and GST activity. Additionally, ontogenetic differences in biomarker activity were detected for blacktips and bonnetheads. Further analyses will incorporate empirical dietary data to discern potential sources of contaminant exposure.

226 Induction of CYP1A and CYP1B in Bottlenose Dolphin Precision-cut Skin Organotypic Cultures Exposed to B(a)P, PCB 77, Crude Oil, and Corexit 9500A

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Organotypic culture is the culture of manually cut or precision-cut organ slices. This is an ex vivo methodology that is applicable to numerous fields including toxicological and pharmacological studies. When compared to cell culture methodologies where one cell type is cultured, benefits of organotypic culture include the preservation and culture of the entire tissue structure with multiple cell types and their interactions present. While organotypic culture is well established in pharmacology and mammalian toxicology studies and known to closely model in vivo processes, it is relatively novel in marine mammal toxicology. In vivo toxicological studies are precluded ethically and legally in marine mammals, thus in vitro or ex vivo studies are needed to understand the cellular responses following contaminant exposure in these species. The skin matrix is suitable for organotypic methodology, and studies have been conducted in cetaceans with this matrix utilizing manually cut organ slices as opposed to the precision-cut methodology reported here. We previously validated this methodology, confirming the viability of precision-cut bottlenose dolphin (*Tursiops truncatus*) skin slices (including blubber) sustained in culture for 24, 48, and 72h with three viability endpoints assessed: (1) cell growth from the cultured organ slices, (2) internal potassium (K^+) concentrations in the cultured organ slices, and (3) lactate dehydrogenase (LDH) activity in media collected from the cultures. After method validation, bottlenose dolphin precision-cut skin slices were exposed to a suite of concentrations of benzo(a)pyrene, polychlorinated biphenyl 77 (PCB 77), or crude oil with and without dispersant for 24, 48, and 72h. Cytochrome P450 1 enzymes were selected as biomarkers of exposure to these contaminants. qPCR analysis of CYP1A1 and CYP1B1 was conducted for each tissue slice using GAPDH, 18S, or YWHAZ as housekeeping genes. Benzo(a)pyrene exposure experiment's preliminary results revealed a statistically significant dose-response of CYP1A1 and CYP1B1 biomarker induction. Further analyses of CYP1A1 and CYP1B1 induction in PCB 77 and crude oil and dispersant exposure experiments are currently underway.

227 Impacts of Crude Oil, PAH and Alkane Exposure on the Intestinal Transport Physiology of the Gulf Toadfish (Opsanus beta)

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Marine teleosts are faced with the continuous challenge of diffusive water loss, primarily through the gills, given their extracellular fluids are hypo-osmotic to the surrounding seawater. To combat this diffusive water loss, marine teleosts continually drink seawater to maintain hydration and excrete excess absorbed salts through their gills. Thus, the gastrointestinal tract of marine fish represents a potentially significant route of exposure to waterborne pollutants such as those found in crude oil, most notably polycyclic aromatic hydrocarbons (PAHs). To investigate the potential osmoregulatory responses of the marine teleost intestine to crude oil exposure, transepithelial short-circuit current (I_{sc}) and conductance were measured in isolated intestinal epithelia from the Gulf toadfish exposed to various dilutions of high energy water accommodated fractions (HEWAF) of *Deepwater Horizon* slick oil using Ussing chambers. Data revealed a dose-responsive decrease in the absorptive I_{sc} with increasing %HEWAF dilution in the anterior intestine, but no significant effects in the middle and posterior intestine. To determine whether specific PAHs (individually or combined) might be responsible for, or contribute to, the observed responses in the anterior intestine, the following 6 PAHs were selected for testing, owing to their relatively high environmental concentrations, persistence and toxicity: phenanthrene, chrysene, pyrene, naphthalene, anthracene and fluoranthene. Additionally, considering that alkanes comprise a much larger fraction of crude oil than PAHs (approximately 4-fold), a mixture of alkanes (C7-C40) were tested. No effect was observed on the anterior intestine electrophysiology using the selected PAHs or alkanes, indicating these constituents alone are not responsible for the decreased ion transport occurring with HEWAF exposure. Finally, the effect of HEWAF on water transport was assessed using isolated intestinal sac preparations. No effect on water transport was observed indicating that the ability to hydrate in the face of HEWAF exposure is likely maintained despite an inhibition of ion absorption.

228 Effects of Subchronic Low Benzo(a)pyrene Exposure on Signaling and Metabolic Pathways in Male Tilapia

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Polycyclic aromatic hydrocarbons (PAHs) including Benzo[a]pyrene (BaP) are widely dispersed in the environment. Contamination in aquatic systems of BaP is of great concern particularly in non-model organisms of economic impact (i.e., tilapia) due to its carcinogenicity and adverse effects on the survival, development and reproduction in fish. In this study we used high-throughput RNA-Seq to investigate molecular responses in the liver and testis of male tilapia following repeated intraperitoneal (i.p.) injection of a low dose of BaP (3 mg/kg every 6 days) for 26 days. The morphometric endpoints were also examined as determinants of general health. After 26 days, the tilapia were sacrificed, and the liver and testis samples were collected for RNA-Seq analysis. The morphometric endpoints including factor condition, hepatosomatic index and gonadosomatic index were significantly decreased after BaP treatment. BaP exposure altered the expression of 1444 genes (607 up and 837 down-regulated) and 309 genes (167 up and 142 down-regulated) in the liver and testis, respectively (p-value < 0.05; fold change $> \pm 1.5$). These genes are involved in many biological processes such as detoxification, enzymes/oxidative stress and metabolism pathways in the liver. In testis, genes regarding immune system, signaling transduction and metabolism were altered. Pathway analysis using Gene Set Enrichment Analysis (GSEA) suggest that in both tissues, the metabolic pathways and cell signaling pathways were altered, i.e., heme oxidation and insulin action. In the liver, specific pathways were affected related to diseases, i.e., thyroid hormones in adipose tissue metabolism. The Gene ontology (GO) analysis revealed potential effects on lipid hydrolysis and phospholipid metabolism process in the liver. In testis, GSEA determined that Intermediate filament polymerization and glycolysis were affected. The GO categories were related to signal transduction and inflammatory response. Recently, it has been reported that disruption in metabolic pathways has a role in carcinogenesis. Therefore, it is suggested that activation of these pathways and the others related to signaling pathways, together with depleted morphometric endpoints, provide new insights to the mechanism of action of chronic exposure to low dose BaP in male tilapia.

229 Behavioral and Physiological Responses of Mahi-Mahi and Bicolor Damselfish to Olfactory Cues Following Crude-Oil Exposure

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The 2010 Gulf of Mexico oil spill overlapped with the habitat of pelagic and reef fishes, including Mahi-Mahi (*Coryphaena hippurus*) and Bicolor Damselfish (*Stegastes partitus*). In fishes, olfactory cues provide essential information about predators, prey, and conspecifics that are crucial to survival; however, the olfactory sensory neurons are directly exposed to the aquatic environment and are therefore susceptible to damage from aquatic contaminants such as crude oil. This study examined the time that control and oil-exposed Bicolor Damselfish spent in a chemical alarm cue compared to control seawater and the time that control and oil-exposed Mahi-Mahi spent in the water-accommodated fraction of crude oil compared to control seawater using a flume choice system. Control Bicolor Damselfish avoided a conspecific chemical alarm cue, while oil exposed conspecifics (32 ug/L for 24-hours) did not avoid the cue ($p < 0.001$). Similarly, control Mahi-Mahi avoided crude oil diluted in seawater, whereas, oil exposed conspecifics (18 ug/L for 24-hours) did not ($p < 0.01$). Following these results, we measured the electrophysiological response of control and oil exposed juvenile Mahi-Mahi to crude oil and prey cues using an electro-olfactogram (EOG). We expect EOG data to show a reduction in olfactory performance with increasing concentration of crude oil. Altered ability to detect olfactory cues could result in reduced survival in these ecologically important species. This research was made possible by a grant from The Gulf of Mexico Research Initiative. Grant No: SA-1520; Name: Relationship of Effects of Cardiac Outcomes in fish for Validation of Ecological Risk (RECOVER).

230 Method Development for Quantitation of Polycyclic Aromatic Hydrocarbons and Their Alkylated Congeners in Organic Tissue by GCxGC-HRTOFMS

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in nature consisting of two or more aromatic rings in their structures, having different source materials. Chromatographic separation of their alkyl-substituted compounds (alkyl polycyclic aromatic hydrocarbons, APAHs) is challenging with present day chromatography. This is due to their vast structural diversity with each alkyl-substituted group possessing many constitutional isomers. This study employs the two-dimensional gas chromatography coupled with a high-resolution time-of-flight mass spectrometer (GCxGC HRTOFMS) to separate and detect APAH congeners. The developed method, done according to EURACHEM guideline, was applied to the quantitation of PAHs and APAHs in a NIST standard reference material (SRM 2974a). Gel permeation chromatography (GPC) was added to the sample preparation procedure after accelerated solvent extraction (ASE) step to aid sample clean-up (lipid removal) thus reducing matrix interferences. The extract was then stored at 4^{o}C in an amber GC vial prior to instrumental analyses. An Agilent 7890 GC equipped with secondary oven, modulator operated at -80^{o}c and a split/splitless injector with a time of flight mass spectrometer fitted with electron ionization (EI) source was used for the MS acquisition. 1 µL was injected with an autoinjector into splitless injector at a temperature of 250^{o}C. The oven temperature was held at 80 ^{o}C for 1 min then raised to 210 ^{o}C at 35 ^{o}C/min, further ramped up to 260 ^{o}C at 3 ^{o}C/min, and finally held for 5 min at 10 ^{o}C/min to 315 ^{o}C. Alkyl PAH standards were then run in full-scan mode from m/z 50 to 350 to select the precursor ion for each homologue with mass accuracy between ± 2 ppm. Selectivity and sensitivity of 16 native PAHs and 18 APAHs was carried out showing linearity of > 0.99 between 5 – 500 pg/µl. The limit of detection (LOD) and limit of quantification (LOQ) of the alkyl PAHs are between 1.23 - 2.21 pg/µl and 4.04 – 7.39 pg/µl. A standard reference material (SRM 2974a), an organic tissue, was used to confirm the developed method. The values obtained from the measured mass fraction of the SRM and the certified mass fraction was then compared. The measurement bias observed was < 25 % for the analytes which falls within the limit/range of acceptance.

231 Environmental Estrogenicity of Water and Sediment within an Agricultural Watershed: Relationship with the Incidence of Testicular Oocytes in Fish

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Environmental estrogens threaten habitat quality and the integrity of freshwater fish populations by altering reproductive behavior and physiology such as induction of testicular oocytes. Municipal and industrial wastewater effluents are often the focus of efforts to characterize environmental risk associated with estrogens; however, major contributors of estrogens in many watersheds include runoff from agricultural fields,

containing animal wastes applied as fertilizer. Chicken litter, often used to amend soil in pasture and row crop operations, contains high concentrations of estrogens which can run off into aquatic systems during rain events. Exposures to environmental estrogens can be enhanced in riverine systems as these rain events and chicken litter applications often coincide with spawning events and sensitive early life stages of fish, posing risk to reproduction and development. Testicular oocytes can be indicative of exposure to estrogens and may lead to population-level effects. To better understand population risks of agriculture-induced endocrine disruption, we compared seasonal trends in surface water and sediment estrogenicity, determined by an estrogen CALUX reporter gene assay, to the prevalence and severity of gonadal malformations among adult male fish from a gradient of agriculture-intensive sites within the Coosa River System (GA, USA). We discuss geospatial and temporal relationships among land use, environmental estrogenicity, gonadal malformations, and documented population declines among imperiled and sport fish species within an agriculturally-dominated basin.

232 Application of a Benthic Impact Model to Assess Potential Risks from Choccolocco Creek Sediment

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A recently-published model (Finkelstein et al., 2017) was used to evaluate the potential for adverse effects to benthic invertebrates in the Choccolocco Creek portion of the Anniston PCB Site (Alabama, USA). The model is based on an empirical relationship between PCBs in sediment porewater and "benthic impact" values derived from a review of effect data from PCB-spiked toxicity experiments in water. The model was used to predict benthic impacts for 33 Choccolocco Creek sediments (with PCB concentrations up to 1185 mg/kg) using measured concentrations of PCBs in porewater and observed effect levels for two benthic test species. The magnitude of benthic impacts were overpredicted compared to observed reproductive effects. Evaluating the observations and model predictions using a community-level effects threshold of 20% indicated that the model performed well for the more sensitive of the two species tested (*Hyalella azteca*). In 19 of 20 sediments in which reproductive effects > 20% for *H. azteca* were observed, the model correctly-predicted a benthic impact > 20%, and in 11 of 13 sediments in which effects < 20% were observed, the model correctly predicted a benthic impact < 20%. The model was then applied to estimate benthic impacts from concentrations of PCBs in porewater predicted from measured concentrations of PCBs in bulk sediment, measured sediment organic carbon content, and site-specific organic carbon-water partitioning coefficients calculated from synchronous measurements of PCBs homologs in porewater and bulk sediment. Model results indicated that concentrations of total PCBs of 1 mg/kg, dry weight (dw) would result in a 2 to 3% benthic impact on reproduction, a negligible level of impact with regards to benthic community health. According to the model results, impacts that could have community-level effects (i.e., > 20%) would not be expected in sediments with concentrations of PCBs of approximately 4.0 to 5.7 mg/kg or less. Concentrations of PCBs in sediments for a majority of Choccolocco Creek rarely exceed these thresholds and support empirical ecological community metrics that confirm healthy benthic communities are present in this system that includes several threatened and endangered mollusc species.

Chemical, Biological and Instrumental Methods for Detecting Harmful Algae Blooms and Their Natural Toxins

233 Monitoring and modeling of harmful algal blooms in Lake Erie and other Great Lakes to protect drinking water and guide mitigation of nutrient sources

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One major issue still plaguing the Great Lakes is the resurgence of harmful algal blooms across western Lake Erie and other Great Lakes embayments. On Lake Erie, progress has been marked by steps taken after the Toledo water crisis of 2014 to bolster monitoring, research, and regulations that affect HABs. Prior to the 2014 incident, we worked with the City of Toledo to conceptualize a state-of-the-science monitoring system that water treatment plant managers could use to monitor lake conditions and raw water quality in real-time, including chlorophyll and phycocyanin pigment sensors. Within a week after the crisis, the concept was realized. Since 2014, the system was expanded to include real-time water quality sensors at 12 public water systems from Toledo to beyond Cleveland in the east. This network of online, year-round, water quality and HABs monitoring sensors is the largest of its kind in the Great Lakes. The network provides a snapshot of nearshore lake conditions every ten minutes and helps protect drinking water supplies for nearly three million people. Research teams have included investigators from the University of Michigan, University of Toledo, Bowling Green State University, Kent State, NOAA, USGS, USEPA and others who are working to advance our understanding of bloom and toxin drivers, and steps that watershed and water plant managers can take to reduce the size, severity, toxicity, and health risks of blooms. The mechanistic Advanced Aquatic Ecosystem Model (A2EM) was recntly used to quantify the internal load of phosphorus that contributes to algal blooms. The model showed that the release of accumulated phosphorus in the sediments can contribute up to an additional 30% of the total phosphorus load from tributaries into the lake each year. This research can help managers assess long term solutions to reduce nutrient loads to the lake. The model was used as part of an ensemble to develop a 40% binational nutrient reduction goal for Lake Erie under Annex 4 of the Great Lakes Water Quality Agreement. This model is also used to inform an annual NOAA prediction in early July of the size of the bloom during the summer peak.

234 Sensing and Educating the Nexus to Sustain Ecosystems (SENSE): Implementation of the Kentucky-West Virginia Partnership

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With the incidence of harmful algal blooms (HABs) increasing nationwide, more resources are directed toward monitoring and predicting bloom locations and severity. Factors complicating these predictions in large river systems are their extension across state and federal regulatory boundaries, multiple land use scenarios, numerous and diverse stakeholders, and multiple designated uses of the resource. Responding to the challenges, Sensing and Educating the Nexus to Sustain Ecosystems (SENSE) is a research program funded by NSF-EPSCoR (2016-2020) that was developed to support cyber-infrastructure development in the partner states. With the specific goal of gaining insight into the influence of food and energy production on aquatic ecosystems and HAB formation, the project has grown and developed through outreach and partnering with stakeholders sharing common goals. Factors influencing HABs often include increased nutrient (N and P) loading from agricultural lands, and elevated temperatures, and decreased flow associated with energy production. Objectives include real-time monitoring of water quality parameters associated with HAB development in Kentucky Lake and the Ohio River with the goals of establishing relationships among water quality and quantity and bloom formation to better predict bloom development and to potentially reduce the incidence of HABs in the targeted water bodies. Aspects of the project implemented in the spring/summer of 2017 have been collection of real-time monitoring data including general water chemistry, chlorophyll a, phycocyanin, nitrates and phosphates that are then relayed to open sources for broad data use. While Kentucky Lake water quality has been monitored for 10 years using high-frequency sensors, finding from the first season of data collection for the new program from both Kentucky Lake and the Ohio River will be reported and will include discussions of stakeholder/partnership development on the large river ecosystems, successes and failures of deployment designs and instrumentation, sampling plans and their modifications, and strategies for moving forward with predictive HAB model development.

235 Transcriptomic analysis of microcystin LR toxicity in the endangered Lost River suckers

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The Upper Klamath Lake (OR) endangered Lost River sucker (*Deltistes luxatus*) populations are threatened by a prolonged lack of substantial recruitment into spawning populations. Despite successful spawning of adults each spring, progeny fish fail to survive past age 1 or 2. A number of factors have been implicated as contributors to low juvenile survival, including poor summer water quality, infectious disease, interactions with non-native species and exposure to the cyanobacterial toxin microcystin. The presence of microcystis cells in the digestive tract of age-0 suckers has been recently observed leading to the hypothesis that ingestion of microcystin leads to increased juvenile sucker mortality. However, histopathology consistent with microcystin toxicity is rarely observed for wild caught suckers from Upper Klamath Lake. Recent immersion-based studies using zebrafish embryos coupled with RTPCR indicated that microcystin LR can lead to endocrine disruption. Endocrine disruption is known to affect development, reproduction and immune status of fish. We hypothesize that ingestion of environmentally relevant concentrations of microcystin found in the Upper Klamath Lake will result in sub-clinical, endocrine disruption events that may impact the recruitment of Lost River Suckers. To address this hypothesis, Lost River suckers raised in a microcystin-free facility were fed diets containing two different concentrations of purified microcystin-LR (36 and 60 ug). After 24 hours, subsets of fish were processed to assess liver and muscle microcystin burden and histology. In addition, four randomly selected fish from each group were sacrificed and gills, intestine, kidney and livers were processed for total RNA. To investigate potential hepatotoxic effects, paired-end libraries were constructed and sequenced to a depth of >40M reads per individual by Illumina HiSeq. A *de novo* assembled reference hepatic transcriptome (150K contigs) was developed and annotated. Current research includes assessing differential gene expression between the two exposure groups versus controls and identifying significantly impacted gene pathways. This includes confirmation of RNAseq using qRTPCR on significantly dysregulated genes, which can serve as molecular biomarkers of microcystin exposure. Overall, this study will provide insight into the molecular effects of microcystin LR ingestion on fish health and how it may relate to reduced recruitment.

236 Developing and applying a novel method for cyanotoxins in water, algal cells, and tissue via liquid chromatography tandem mass spectrometry

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Blue-green algae, or cyanobacteria, are prokaryotes that were among the first photosynthesizing organisms on Earth. Under the proper conditions, cyanobacteria grow and can form dense blooms that also produce harmful secondary metabolites known as cyanotoxins, which pose significant health risks to humans and animals through multiple exposure pathways. Identifying toxin production by cyanobacteria present in water, algal cells, and fisheries is significant to ensure the high quality of food and water for consumption and recreation. The current literature discusses many methods for the detection of cyanotoxins. However, many of these methods only detect discrete classes of cyanotoxins in one matrix (tissue, water, or algal cells). Furthermore, these methods do not include saxitoxin, a select agent, which several freshwater species of cyanobacteria have been documented to produce. Thus, the present study proposes development and application of a multi-matrix (water, algal cells, tissue) screening method for actively understanding cyanotoxin occurrence and bioaccumulation in lakes and reservoir systems. For the proposed methods chromatographic separation was carried out using an Agilent 1260 Infinity HPLC interfaced with an Agilent G6420 Triple Quadrupole Mass Spectrometer. Due to inadequate separation and retention of saxitoxin using just Reverse Phase Chromatography (RPLC), two separate methods were used to separate toxins for quantification. The first method achieves separation using Hydrophilic Interaction Chromatography (HILIC) (anatoxin-a, saxitoxin, and cylindrospermopsin) on a TSKgel Amide-80 HILIC column. The second method achieved separation using RPLC (microcystin – LA, LY, LR, LF, YR, RR and nodularin) on a Poroshell SB-C18 RPLC column. Two solid phase extraction methods were chosen to isolate cyanotoxins from water. The Waters Oasis HLB was chosen for microcystin – LA, LY, LR, LF, YR, RR and nodularin, and the Supelclean ENVI-carb for anatoxin-a, cylindrospermopsin, and saxitoxin. Lyophilized tissue and algal cell matrix were extracted with different liquid mixtures of MeCN, MeOH, 0.3% acetic acid, or 0.1% formic acid to evaluate the best extraction mixture for each matrix. Method detection limits, extraction efficiencies, and calibration linearity were evaluated for method validation. The application of this method within a eutrophic reservoir will be used to study bioaccumulation and trophic interactions of cyanotoxins.

237 Measuring Total Microcystins in Fish Tissue using the 2-methyl-3-methoxy-4-phenylbutyric acid (MMPB) procedure

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There are limited methods for the analyses of multiple algal toxins in aquatic food webs, phytoplankton, zooplankton, periphyton, macroinvertebrates, forage fish, bottom feeders and top carnivore fish. Algal toxins in freshwater systems do not necessarily occur as single contaminants; mixtures of toxins may be produced from Cyanobacteria, *Prymnesium parvum (Prymnesins)*, and *Euglena sanguinea*, including microcystins, saxitoxins, cylindrospermopsin, anatoxin-a., prymnesins and euglenophycin. This can be challenging when the chemical properties of toxin variants complicate the use of a single extraction method. The objective of the first phase of this research was to spike existing fillet and whole fish homogenates with 3 congeners of microcystins (LR, LA and RR) individually and as mixtures, and to develop a method for their recovery and measurement using the MMPB derivatization technique. The second phase of the project is to field-test this method on fish collected from water bodies experiencing algal blooms and compare results with individual congener measurements. Extraction methods and analytical methods being developed for this research will be a starting point for developing extraction procedures for plankton, periphyton, and macorinvertebrates. Ten and 100 mg of fish homogenates from fish containing 1, 4 and 14% lipids were spiked with 4 and 40 ng of each of the microcystin congeners, LR, LA and RR. Various extraction techniques and conditions were tested to optimize recovery and simplify the procedure. Overall toxin recoveries were found to range from 30 to 50% based on spike concentrations, and were not significantly impacted by the lipid content. The lipid content did impact the workup/extraction procedure in ways which were accountable through the use of a surrogate standard, allowing for accurate evaluation of microcystin concentrations.

238 Advanced high resolution accurate mass acquisition modes for non-targeted and unknowns analysis of microcystins

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Microcystins are cyclic heptatpeptide hepatotoxins produced by certain species of cyanobacteria (blue-green algae) found in freshwater environments. These secondary metabolites are toxic to higher organisms, causing human sickness or even death in some cases. There are only a handful of microcystin standards available in the market and over 90 different microcystins variants have been reported. For this reason, it is important to develop non-targeted methods for the analysis of these compounds. An automated method for microcystins extraction and clean up from water samples was developed using 2D-LC in the *trap and elute* configuration: a large volume of water sample (500 µl) was directly injected and trapped in the first column. After that, microcystins were desorbed in reverse flow and injected to the analytical column, prior to mass spectrometry analysis. When the QTof-MS was operated in high resolution full scan mode (RP ≈ 25,000), the instrument proved to be very sensitive for microcystin-LR (50 fg on column with S/N > 10). Combined with the 2D-LC, the system could detect 100 pg/L of microcistyn-LR in water with S/N>10. Mass accuracy (< 1 ppm) allowed assigning elemental compositions for unknown compounds with confidence. MS/MS mode monitoring the characteristic microcystin ion at m/z=135.0804 was useful to provide quantitative results of targeted compounds in complex samples. Advanced acquisition methods such as Data Dependant Acquisition (DDA) and Data Independent Acquisition (DIA) were successfully employed to elucidate new microcystin variants (1044 & 1071 *m/z*) in real samples that have not been reported in literature yet.

239 Two Novel Passive Sampling Devices with Nanopore Sorbents for Monitoring Microcystins and Other Toxins in Water

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A major challenge to the quantitative risk assessment of microcystins produced from cyanobacteria blooms is determining chronic exposure to the toxins. The USEPA recently issued 10-day exposure health advisories for microcystins, but concentrations in water can vary several orders of magnitude over a 10-day time period due to the complexities of bloom formation and persistence, and toxin production, extracellular release, transport and fate. We have developed 1) a fiber passive sampling device (fPSD) and 2) a non-selective passive sampling device (nsPSD) to measure the time-integrated concentrations of microcystins in water that overcomes the challenge of measuring the chronic exposure over a 10-day period. The fPSD contains nanopore sorbent particles packed in a porous hollow fiber that allows the microcystins to pass through the membrane and be sorbed and preserved within the nanoporous particles. The nsPSD contains similar sorbent but within a non-selective copper mesh that also allows microcystins and other natural toxins to pass through to the sorbent. The nanopore sorbent prevented the accumulated microcystin-LR (MC-LR) from degradation for 25 days, demonstrating preservation of the sorbent-bound MC-LR. In contrast, MC-LR was completely degraded within 20 days in lake water. Both PSDs remain in a linear uptake phase for at least 15 days, exceeding the USEPA 10-day exposure requirement. The sampling rates of the fPSD and nsPSD were 0.017 L/day/cm^2 and 0.045 L/day/cm^2 at an exposure concentration of 0.1 µg/L MC-LR. The fiber format is self-supported, requiring no external frame for fabrication, whereas the nsPSD is held within a supporting frame. A higher sampling rate can be achieved simply by using multiple fPSD fibers or a larger nsPSD format. Both PSDs can be used for environmental monitoring of microcystins well below regulatory thresholds (sub nanogram-per-liter), have a low fabrication cost, are easy to deploy and recover, and are compatible with any common method of detection. Field verification that both PSDs provide comparable 10-day exposure estimates to repeated daily sampling over a 10-day period was performed in Dianchi Lake, China. Furthermore, the nsPSD appears to also work well for other natural toxins such as brevetoxins.

240 The Detection of Cyanobacteria, Algae, and Picoplankton using Flow Cytometry, Morphology and Hyperspectral Imaging

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The detection of cyanobacteria algae, and picoplankton, in water is an important step in assessing water quality. Studies were initiated using fluorescence microscopy, flow cytometry and hyperspectral imaging with two fresh water species that were cultured in the laboratory: *Microcystis Aeruginosa* (cyanobacteria), and *Selenastrum capricornutum (*algae). Cyanobacteria were distinguished readily from algae by fluorescent microscopy that selectively excited different photosynthetic pigments in the two species with blue or green fluorescent light. The cyanobacteria are brighter with green light excitation while algae are brighter with blue light excitation. By sequentially combining microscopic images from these two excitation wavelengths, organisms can be quickly classified as either algae or cyanobacteria. The positive identification of each organism was then confirmed through its emission wavelengths using the Prism and Reflector Imaging Spectrometer System (PARISS, hyperspectral imaging system). The mean fluorescence emission from algae is about 25 nm longer than from the cyanobacteria. The Stratedigm flow cytometer can count 1000 organisms per second. This provides statistically accurate data in distinguishing large quantities of algae, cyanobacteria, and picoplankton. Their identification was based on their size and selective intensity emissions following excitation by a blue (488 nm), yellow (550 nm) or red (640 nm) lasers Fluorescence from algae and cyanobacteria are observed in all detection channels with these lasers. However, the intensity of cyanobacteria emission following yellow or red laser excitation was preferentially detected in different channels from those of the algae. Likewise, the emission intensity from algae was detected in different channels after excitation with the blue laser. Thus the organisms could be detected by viability size and selective fluorescence emission. Flow cytometry techniques developed with the laboratory cyanobacteria, and algae were then applied to study lake picoplankton which had a size between 1-2 um. New methodologies to study photosynthetic single cell organisms are described that can be useful to identify and distinguish cyanobacteria from algae and healthy from dying organism. In the future these techniques can be applied to field studies in streams and lakes This abstract does not represent USEPA policy.

Implementing the Three Pillars of Sustainability in Assessment and Decision-Making

241 Incorporating Stakeholder Values into Sustainable Remediation: It's all Social

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All remedy decisions must balance trade-offs to demonstrate, in terms of environmental, economic and social indicators, that the benefit of undertaking remediation is greater than its impac. Regulatory decisions should consider affected communities' needs and values, and how these might be impacted by options; this process requires that diverse stakeholders are able to engage in a transparent consideration of value trade-offs and of the distribution of risks and benefits of remedial actions and outcomes. Although guidance has been progressing, the focus remains on green remediation, or footprint reduction, rather than a full consideration of sustainability. The Sustainable Values Assessment (SVA) tool was developed to link environmental quality, economic viability and social equity metrics to a range of stakeholder values. Metrics are developed, scored, and aggregated, and remediation alternatives are ranked in terms of stakeholder group (SG) priorities. The SVA has two main components: 1) Technical scoring, in which remedial options are scored based upon metrics of their impacts (desirable and undesirable) on stakeholder values, and 2) Social weighting, in which values are weighted based upon the priorities of relevant stakeholder groups. To score options, metrics of stakeholder values are evaluated in terms of the technical aspects (e.g., time, volumes, footprint) of a remedial alternative. This provides quantitative, comparable scores for each alternative based upon their environmental, economic and social impacts. Weights consider the relative important of different impacts to various stakeholder groups. Using the SVA calculation tool, values are scored and weighted; the relative sustainability of cleanup options under consideration are thus evaluated in terms of differing community priorities. This integrates data from larger projects assessing environmental and economic aspects of sustainability, going well beyond the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) 9 criteria for evaluating remedial options and allows for the communication not only of traditional sustainability "scores" for remedial options, but also how options might be ranked given the diverse values and priorities of stakeholders, who might otherwise focus on single or narrow issues. This approach identifies trade-offs and points of potential contention, providing a systematic, semi-quantitative, transparent valuation tool for community engagement.

242 Sustainable Remediation of Solid Waste: How to Nip it in the BUD

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Beneficial Use Determinations (BUDs) are an underutilized but proven method of executing remediation and construction projects that are both sustainable and cost-efficient. As part of a large-scale resiliency effort, thorough and proper management of sediments generated from the dredging of a tidal wetland and upstream lake were found to be compatible for reuse within a similar wetland habitat. This approach is particularly effective for sites where the cost and carbon footprint for off-site landfill disposal of solid waste are immense and can be accomplished on all types of sites looking to dispose of or acquire fill material. Through strategic planning and negotiations with State regulators, the somewhat intimidating beneficial reuse process can be made quite effortless before it develops into an issue on an active work site. This presentation demonstrates the experience gained from the successful planning, design, and implementation of beneficial reuse concepts on a multi-phase drainage improvement and wetland restoration project in a neighborhood that previously suffered from chronic flooding conditions and poor water quality. Results discussed from this one project will include the volume of material averted from landfills, cost savings from avoiding off-site disposal and reducing imported fill volumes, and reductions in transportation mileage, fuel consumption, and carbon production. The volume of material approved to be beneficially reused was over 40,000 cubic yards, providing an overall project cost saving of approximately $4 million and prevented the emission of over 1.9 million pounds of CO_2. The benefits of this project will portray the importance of understanding the means of obtaining approval under applicable regulations, thoughtfully managing material excavation, transportation and end-use, and aligning the schedules of multiple client projects for a seamless transition and successful reuse of solid waste.

243 Climate Implications of Feedstock Agnostic Biorefineries

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Based on the resource estimates in the 2016 *Billion Ton Study*, nearly 95% of all currently-existing biorefineries will have poplar, corn stover, and *Miscanthus* available within a 60-mile radius, should they be retrofitted to utilize lignocellulosic feedstocks. Any facility within reasonable distance of residential areas will also have access to more heterogeneous municipal solid waste, wastewater treatment sludge, and other commercial and/or industrial organic waste. However, many of the advanced conversion technologies available for valorizing these feedstocks cannot handle a diverse set of feedstocks. Research and development focused on testing different feedstock blends does not necessarily pursue the feedstock blends most likely to be available, which depends on sub-county-level geospatial distributions of crop production, harvesting timelines, feasibility of long-term feedstock storage, and the use of crop rotations. For biorefineries capable of utilizing different feedstock blends, benefits may come in the form of lower delivered costs, reduced transportation times and distances, diminished storage losses, and lower life-cycle greenhouse gas (GHG) emissions. We present the first sub-county-level analysis of bioenergy feedstock blending in the U.S., based on both the *Billion Ton Study* 2016 data, and NREL's Bioenergy Atlas data, aimed at elucidating which blends are most valuable from an economic and climate standpoint, and how sensitive those results are to different assumptions about biomass availability and biorefinery performance. Our study provides insight into how pretreatment technologies, including ionic liquid pretreatment, can be best applied on a local scale to handle individual feedstocks or expected blends, and what additional research would yield the most significant potential reductions in biorefinery costs (and thus competitiveness), GHG emissions, and stability of feedstock supply. We also show which feedstock blends are likely to be more amenable to thermochemical conversion, and which blends warrant further research on biochemical conversion platforms.

244 Using linear alkylbenzenes to identify anthropogenic contributions to aquatic pollution in complex ecosystem: Case study of PRD, China

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Identifying contaminants inputted to the environment and tracing anthropogenic contributions from various socio-economic activities are desirable for implementing effective management and remediation strategies. Linear alkylbenzenes (LABs) have been used as molecular markers to identify the potential anthropogenic origins for other organic pollutants with similar physiochemical properties, such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). Linear alkylbenzenes are usually discharged in the environment as residues of commercial detergents and closely related to socio-economic activities during urban development. The different sources of LABs were investigated in this framework, with the use of factor decomposition method and experimental data. The cities located in the Pearl River Delta with one of the highest population densities and fastest economic growth rates in China are mainly responsible for production and discharge of aggregated pollutants in the aquatic system. The framework identified the direct (including urban and rural domestic sewage and industrial wastewater) and indirect contaminant sources (including atmospheric deposition and agricultural irrigation). The results are indicative of how different socio-economic activities contribute to aquatic pollution and which sectors in urban systems are responsible for the pollution. The sources of other organic pollutants will be discussed by applying the framework and comparing with the percentage of LABs. Identification of environment pollution liability is an important part of integrated pollution control in complex ecosystem. The current research provides theoretical support and a database for the formulation of policies leading to regional organic pollutant reduction and remediation of pollution.

245 Translating the -omics toolbox to sustainability solutions

D.L. Carr, *Texas Tech University / Biological Sciences*

In recent years the toolbox available to evaluate environmental impact and restoration potential has expanded to include metagenomic and metaproteomic approaches. We examine the potential for streamlined bioanalytical methods to evaluate function to inform remediation strategies and monitor restoration of soils and sediments. Microbial diversity and functional redundancy are the basis of higher levels of ecological organization and can predict long term success of restoration efforts. Whole microbial community genomics and proteomics are useful in moving beyond descriptive science and have utility in determining if perturbation events require in depth remediation or less costly, support services. Specific selected studies are presented to define how a hypothesis driven approach can be used to confirm that specific microbial mediated functions are present to mediate the effects environmental perturbations.

246 Remedy and Recontamination Assessment Array: An Initial Proof of Concept

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Costs for design and implementation of remedial alternatives for contaminated sediment sites are considerable. While pilot scale demonstrations offer a means to establishing efficacy of proposed remedies under site-specific conditions they are also resource intensive. Thus, there is a need for a more cost-effective and timely approach to establish likelihood of success of proposed remedial alternatives under site-specific conditions prior to pilot or full scale implementation. The research presented here describes one such approach to establish efficacy of proposed remedies and identify potential sources of ongoing contaminant input to better inform remedy selection, design, implementation, and monitoring directly within an area of concern. The project is a proof-of-concept for a remedy and recontamination assessment (RARA) array providing, direct, site-specific, measurement of recontamination potential and impact on a range of remedies. The RARA is designed to increase exposure realism relative to laboratory treatability studies at a reduced cost and complexity compared to large-scale pilot studies. Development and testing of the RARA array included: (1)Conceptual design of the array and field methodology; (2)Construction of the prototype RARA array and testing of the methodology; (3)Proof-of-concept field deployment of the RARA array, and (4)Evaluation of the performance and feasibility of the method. Using the RARA array and methodology, we conducted a proof-of-concept deployment with contaminated sediments collected from a Navy sediment site (Chollas Creek, Naval Base San Diego) that is being investigated under the Total Daily Maximum Loading (TMDL) program. Sediments were tested as untreated or treated with thin-layer of clean sand or clean background sediment from a reference area. The RARA was placed back in the bay and monitored for 5 months. The system incorporated sediment traps around the perimeter of the array to capture depositional sediments. The RARA also incorporated current profiling, turbidity, and water quality sensors and passive samplers to monitor conditions during the deployment. The system allows for a range of measurement capabilities to provide the basis for the assessment or remedy effectiveness and recontamination including chemical analyses, benthic community assessment, and bioaccumulation. The concept of source influence on the remedies as well as the performance of the two sediment treatments were successfully demonstrated.

247 Assessing the potential contributions of the microbial communities occurring within concrete to environmental processes in built landscapes

J. Brown, *D.L. Carr, Texas Tech University / Biological Sciences*

Concrete made with Portland cement is the most widely used man-made material. This man-made rock is accumulating worldwide and is a fundamental feature of urbanized and human-dominated landscapes. Yet, it is rarely considered as a biologically active component of the biosphere. We hypothesized that the total volumes of concrete, including the internal cracks and pore spaces, are habitat to diverse microbial communities, and that these communities, due to the vast quantities of concrete, are performing significant ecosystem-level functions. Preliminary analyses using replicated PCR-amplified 16s gene sequencing detected 228 bacterial genera embedded inside a sample of solid Portland-cement based concrete. Scanning electron microscopy (SEM) sample visually confirmed several clusters of intact microbial cells existing in microscopic internal crevices. This was expected likely given that microbial communities consisting of bacteria, fungi, and algae are known to live on the surfaces of concrete and inside natural rock, where they alter geochemical cycles. We analyze the composition microbial communities in a variety of concrete samples with SEM and sequencing of 16s and 18 rRNA genes (to assess both bacterial and fungal diversity). Microbial activity is quantified by periodically measuring CO2 respired from concrete samples in sealed containers with an infrared gas analyzer. Community-level physiological profiles regarding potential carbon metabolism are generated for each sample via Biolog™ EcoPlate systems. Together, these provide baseline information regarding the functional and biological diversity of these incidental and overlooked communities. Basic biological analyses provide the basis for quantifying ecosystem services and environmental processes that these “endo-concrete” microbial communities, contribute to the sustainability assessment of the built environment.

248 Modeling Indoor Occupational Air Emissions of Nanomaterials for Life Cycle Assessment

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Engineered nanomaterials (ENM) provide industrial and commercial benefits across many sectors but they also raise concerns over their potential environmental and human health impacts. Throughout the life cycle of a product, occupational human health impacts are particularly pertinent given the constant interaction workers may have to large volumes of nanopowders. Neglecting such impacts could result in the burden shifting from the environment to workers. However, there are certain limitations in life cycle impact assessment’s (LCIA) ability to evaluate the direct impacts from emissions of ENM themselves as data and models in the literature that describe the fate, exposure, and effects of ENM are limited. Here we present a two-zone, dynamic fate and transport model for use with indoor, occupational ENM airborne emissions. The fate and transport model is linked to a physiologically based pharmacokinetic inhalation exposure model that considers the mucociliary clearance, phagocytosis, and translocation of particles into the systemic circulation. Using the case-study of nano-TiO_2 emissions in the indoor workplace, nano-specific life cycle assessment characterization factors (CF) are presented. Near-field concentrations of a steady-state model is roughly 90% lower of the maximum concentrations calculated by the dynamic model. Inhalation exposure and final retention of particles in the lung was significantly influenced by the magnitude of the airborne concentration. As airborne concentrations rose, phagocytizing cells in the air-exchange and interstitial regions became saturated. The dynamic model presented here resulted in an inverse relationship between the total indoor air emissions of nano-TiO_2 and the resulting magnitude of the CF. This is due to the final retained intake fraction (RiF), whereby smaller emission levels lead to greater fractional deposition and retention in the lung compared with larger emissions. Thus, the CF intended for quantifying the human health impacts resulting from the inhalation of indoor air emissions to ENM may be emission-magnitude dependent. The results of this study demonstrate the importance of applying non-steady state modeling for the estimation of fate and exposure of ENM in the occupational indoor setting.

Environmentally Relevant Behavior Assessment to Support Modeling, AOPs and Improved Risk Decision-Making

249 Linking behavioral effects of methylmercury to adverse population outcomes in yellow perch using an individual based model

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Yellow perch populations in Lake Michigan declined in the 1990s which was attributed to consecutive year-class failures following changes in trophic structure and adverse habitat conditions. The potential effects of contaminants on yellow perch recruitment have not been explored fully either in isolation or in combination with other stressors. Methylmercury (MeHg), a persistent contaminant found in the Great Lakes, has been shown to alter foraging and predator avoidance behaviors of larval yellow perch, which could affect their recruitment. We adapted a larval fish individual based model (IBM) to incorporate laboratory derived MeHg behavioral effects on a larval yellow perch. Effects on swimming speed and foraging efficiency simulated during a 0.21, 0.95 and 3.14 µg/g total mercury (THg) whole embryo tissue exposure resulted in a 13.8 %, 87.7 %, and 64.6 % decrease in survival in a Lake Michigan larval yellow perch cohort, respectively, relative to a MeHg absent simulation. While our experimental THg concentrations were higher than those typically found in Lake Michigan, our findings suggest that exposure to neurotoxic contaminants, such as MeHg, when coupled with low food availability and suboptimal water temperatures, could be an additional factor impacting recruitment of yellow perch in Lake Michigan, and that this deserves further exploration.

250 Environmentally relevant 2,4D exposure reduces baseline aggressive behavior crucial to social contest dynamics in a simultaneous hermaphroditic fish

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The mangrove killifish (*K. marmoratus*) is an indicator of environmental pollution in wild mangrove habitats but little is known regarding their behavioral responses to environmentally relevant doses of herbicides. This is of concern given the generous utilization of herbicides in proximity to mangrove forests in the state of Florida, specifically 2,4-Dichlorophenoxyacetic acid (2,4D). Freshwater fishes dominate 2,4D exposure studies, and seldom investigate environmentally relevant aggressive behaviors crucial to survival. Using *K. marmoratus* as a toxicological model, this novel study aims to understand genetic influences on hormonal mediation of both aggression and social contest dynamics in this species after herbicide exposures. To accomplish this, I analyzed flexibility in aggressive and hormonal responses in fish exposed to a control solution (ethanol) or experimental solutions (a low dose, 3 milligrams/Liter; or high dose, 6 milligrams/Liter of 2,4D dissolved in ethanol) and these were added to the same tank water daily for 9 days. Hormone samples and baseline aggression tests were performed pre-and-post exposure. Baseline aggression was assayed using a mirror test (individuals exposed to own reflection in a mirror). Statistical analyses show no replicate effect and no interaction between treatment and replicate. I found an overall decrease in behavioral expression of aggression with 2,4D treatment. In the high dose group, post-exposure total aggression was predicted by 2,4D exposures; when treated with a high-dose, fish experienced a significant decrease in total aggression. In addition, individuals with longer body lengths displayed increased total aggression (though, still significantly decreased comparatively to controls). Changes in aggression explained by 2,4D treatment raise questions regarding neuroendocrine mechanisms modulating behavioral changes. Individuals exposed to higher doses exhibited decreased aggression, and previous research suggests this may be associated with lower levels of cortisol. Influences of body size on behavior expression suggest that in larger individuals, testosterone production may have been impacted by 2,4D exposure, however, hormone samples have yet to be analyzed. It is interesting to speculate but important to note that 2,4D was not quantified in tank water, therefore, it is not possible to say whether 2,4D itself or degradation products of 2,4D, such as 2,4-Dichlorophenol, influenced behavioral outcomes.

251 Intraspecific genetic variation for lead-induced changes in reproductive strategies

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Little is known regarding genetic variability for behavioral sensitivity in response to anthropogenic lead (Pb) exposure, particularly for behaviors that are essential for fitness and population persistence. Our aim was to identify intraspecific genetic variation in the response of reproductive behaviors (e.g. mating and fecundity) to Pb exposure, using a model system *Drosophila melanogaster*. To accomplish this, a subset of the *Drosophila* Genetic Reference Panel (DGRP) inbred lines were reared on control or leaded (500 µM PbAc) medium from egg stage to adulthood and tested for line and treatment differences in copulation latency (time between pairing and copulation in minutes), copulation duration (length of copulation in minutes), and fecundity (total number of sexually mature adults per female). Pb influenced intraspecific genetic variation in copulation latency, copulation duration, and fecundity ($P < 0.05$). Pb exposure significantly decreased fecundity across DGRP lines. We found significant genotype-by-environment interaction for copulation duration, indicating a genotype-specific response to Pb treatment (either increase or decrease in duration). Genetic correlation matrices revealed significant genetic correlations ($P < 0.05$) between control and Pb-treated flies for mean copulation latency, copulation duration and fecundity. Approximately 50-60% of the genetic variation mediating Pb-induced responses were similar between the treatment groups, but the moderate genetic correlations between the treatments indicate that there is additional genetic variation because of Pb-exposure. These results emphasize the importance of considering the impacts of intraspecific genetic and phenotypic variation in susceptibility to Pb pollution on behavioral phenotypes important for individual-level fitness.

252 Using larval zebrafish (Danio rerio) to screen emerging pharmaceutical pollutants for altered swimming behavior

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Pharmaceuticals are a diverse group of compounds that are increasingly detected in surface waters around the world as their development and consumption increases. The relatively low concentrations found in the environment are not likely to present a risk for acute toxic effects in aquatic organisms, however sublethal exposures may alter normal activity in ways that could impact survival. Behavior is a powerful tool in assessing potential impacts of such pollutants as they are sensitive indicators of sublethal effect, are easily measured through automated tracking software, and are key to an individual's survival and fitness. We have developed a larval zebrafish (*Danio rerio*) assay to screen pure pharmaceuticals and environmental samples for behavior-altering potential, including changes to visual motor response (VMR), acoustic and visual startle response, and stimulus habituation. We have screened 16 different pharmaceuticals for behavior altering potential, including several classes of antidepressants (and their metabolites), antipsychotics, anticonvulsants, sedatives, and an opioid painkiller. Screens on waste water effluent and receiving water samples, as well as simple mixtures of pure pharmaceuticals, are currently underway. Results to date have identified a diversity of antidepressants and their metabolites (amitriptyline, fluoxetine, paroxetine, venlafaxine, sertraline) and a typical antipsychotic (haloperidol) with behavior-altering activity. VMR was the most sensitive endpoint measured, showing a 38-41% reduction in swimming activity with exposure to various pharmaceuticals. Only one compound (amitriptyline) caused a significant, but small, increase in acoustic and visual startle response, while no compounds significantly altered habituation. We intend to incorporate the data from these screens into more complex behavioral investigations assessing prey capture and learning, which ultimately will be used to parameterize population level models. Behavioral data, such as those presented here, will allow scientists and managers to better predict contaminant impacts to natural systems.

253 Systematic evaluation of flame retardant neurodevelopmental toxicity in a multidimensional high throughput zebrafish system

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The heavy use of flame retardant chemicals in many consumer, commercial, and industrial products has led to increased potential for human exposures. There is a great degree of uncertainty regarding the neurobehavioral hazard or risk posed by individual flame retardant chemicals in this highly diverse group of commercially important chemicals. In order to evaluate, and directly compare, the biological activity of a diverse collection of 61 flame retardants (procured from various sources: 43 from the US Environmental Protection Agency – National Center for Computational Toxicology (US-EPA-NCCT), 2 from the NIH National Toxicology Program (NTP) (Pentabromodiphenyl ether and Perfluorobutanesulfonate) and 16 purchased commercially from Sigma) we used a powerful morphological and behavioral testing platform in zebrafish. Zebrafish were exposed to flame retardant chemicals (FRCs) from 6 to 120 h post fertilization (hpf) across concentrations spanning 4 orders of magnitude (eg, 6.4 nM to 64 µM). Flame retardant effects on survival and development were evaluated at 24 and 120 hpf, and neurobehavioral changes were measured using two high throughput photomotor response (PMR) assays. Distinct patters of biological activity were identified for the FRCs and these activities generally clustered by chemistry type. A subset of these FRCs were used to determine the consequence of developmental exposures on adult behavioral responses. Adult behavioral assessments included assessments of social, fear, and anxiety phenotypes. Using novel ToxPi data analysis and visualization tools, these zebrafish responses to FRCs were compared to the USEPA ToxCast results for 42 individual FRCs. These data are being used as a basis for the selection of representative FRCs for transcriptomic analysis. This presentation will provide further evidence that zebrafish neurodevelopment is highly sensitive to many FRCs currently in use and can be used to discover adverse outcome pathways and to better understand potential vulnerabilities to human health.

254 Olfactory neurobehavioral injury as an adverse outcome pathway for environmental neurotoxicants

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Fish rely on olfaction to maintain essential neurobehaviors such as predator detection and avoidance, prey selection, social behavior, imprinting, and habitat location. Salmonids, in particular, have a well developed sensory system that relies on olfactory detection of chemical cues in the environment for these behaviors as well as for outmigration and homing to natal streams. However, exposure to environmental neurotoxicants, including certain trace metals and pesticides, can disrupt these behaviors leading to a loss of survivorship. Ten years of study in our laboratory using two species of fish (coho salmon and zebrafish) have shed light on the molecular mechanisms, adaptive responses, and physiological outcomes associated with chemical inhibition of olfactory function. Specifically, environmental neurotoxicants can cause early changes in the redox status of the olfactory system associated with oxidative stress. By contrast, the fish olfactory system has a robust NrF2-mediated antioxidant protective pathway that can mobilize protective gene responses to respond to adverse changes in oxidative stress. Analysis of salmon promoter flanking sequences for several genes important in mitigating metal injury to OSNs have informed our Nrf2 studies and have facilitated biomarker development. Other early events in olfactory injury include modulation of G-protein coupled receptor signaling, followed by alteration of olfactory sensory neuron populations and induction of cellular apoptosis in the olfactory rosettes. Whereas rapid recovery from copper-mediated olfactory injury can occur in salmon and zebrafish, exposure to cadmium can cause long-lasting deficits in neurobehavior. Collectively, our studies as well as those from other laboratories have provided linkages among early initiating events, cellular and physiological responses, and whole animal phenotypic changes associated with loss of survivorship that provide the conceptual basis for olfactory-mediated neurobehavioral injury to as a relevant adverse outcome pathway for regulatory decisions that support protection of aquatic species. Supported by NIEHS Superfund P42-04696.

255 Using quantitative neurobehavioral assays to develop a multi-species larval fish AOP

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Developmental exposures to high concentrations of neurotoxicants such as methylmercury (MeHg) and PCBs elicit devastating effects to motor and cognitive functions. However, the neurotoxic effects of lower, more ecologically relevant, exposures are much more difficult to recognize as they often yield subtle changes in cognition and/or behavior. As larval fish are particularly sensitive to predation and starvation, these subtle changes can have drastic effects on the survival of the individual and, ultimately the fitness of the population. We are working to develop ecologically-important behavioral assays to better link assessments on individuals to population-level outcomes, improve modeling efforts, and enhance the development of Adverse Outcome Pathway (AOP) models that link molecular initiating events to population-level impacts. We are using quantitative neurobehavioral assays for relatively simple behaviors (*e.g.*, swimming, prey capture and additional assays currently under development), to assess the effects of developmental exposure to MeHg and PCB126 in the zebrafish model system (*Danio rerio*) as well as two ecologically relevant species, the fathead minnow (*Pimephales promelas*) and the yellow perch (*Perca flavescens*). We are exploring molecular initiating events induced by MeHg and PCB126 exposure via comparative RNAseq analysis of larval brains from each species. Collectively, these neurobehavioral and transcriptional data will be used to inform population models and predict long-term population impacts for all three species, as well as to generate a larval fish AOP with cross-species extrapolation capabilities that will allow for population-level predictions on ecologically relevant species from data generated using typical laboratory model species.

256 Examining how contaminant effects on fathead minnow behavior impact cohort growth and survival using an individual-based model

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Methylmercury and PCBs have been shown to negatively affect swimming speed and foraging success in larval fishes. These nonlethal effects on fishes can translate into reduced survival due to increased risk of predation and decreased growth rates in young fishes. We developed an individual-based model (IBM) to determine how impacts of methylmercury and PCBs on larval fathead minnow (Pimephales promelas) fish swimming speed, handling time, reactive distance and capture success would impact larval growth rates and survival. The IBM followed a cohort of larval fathead minnows from hatch through the first 100 days of life. Each day, individual larvae forage, grow, and experience predation and starvation mortality. Contaminant-induced impairments on learning measured in laboratory behavioral assays were incorporated into the foraging subroutine via changes in swimming speed, handling time, reactive distance and capture success. We hypothesized that effects on larval fish behavior, through reductions in foraging success, would result in significantly slower growth rates and reduced survival than in scenarios in which contaminant-impairment was not affected. Model results highlight the importance of considering nonlethal effects of toxicants on fish behavior and how these can affect fish cohort survival and, potentially, population dynamics. In the future, we will incorporate effects of these two toxicants on other species, including zebrafish, to determine if model organisms can be used to determine impacts of toxicants on ecologically relevant and diverse species.

Integrated Tools for Improving Environmental Fate and Risk Assessment for Unregulated Contaminants and Their Mixtures – Part 1

257 In Silico Prediction of Bioremediation Potential

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In 1995, we developed the public resource known as the University of Minnesota Biocatalysis/Biodegradation Database (1). It is currently the EAWAG Biocatalysis/Biodegradation Database, maintained in Switzerland and still freely available to the public. The utility of the database was ienhanced in 2004 with the introduction of the pathway prediction system (PPS) (2). The PPS uses computational metabolic rules for the prediction of microbial biodegradative pathways for known and newly synthesized chemicals. Most recently, these prediction tools have been augmented with a system called RAPID. The RAPID computational tool is being developed to identify enzymes and bacteria capable of biodegrading difficult-to-degrade compounds. Most recently, the system has been focused on personal care products, such as the pharmaceutical carbamazepine (3), and polycyclic aromatic hydrocarbons (4).

258 High-Throughput Effect-Directed Analysis: A novel platform for rapid and sensitive identification of toxic compounds in the aquatic environment

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All kinds of emerging contaminants, including pharmaceuticals, endocrine disrupting compounds and perfluorinated compounds, are present in the aquatic environment, wastewater and drinking water sources. In addition, numerous bioassay surveys have demonstrated the presence of not yet identified compounds with toxic activities in the environment, which is evidently of high concern. For example, genotoxic and endocrine disrupting compounds may lead to DNA damage, to disruption of development and reproduction and/or to cancer. Generally, identification of toxic compounds is done by effect-directed analysis (EDA). In this approach, complex environmental samples showing toxic activity are fractionated with liquid chromatography into a limited number of fractions. Each fraction is tested for toxic activity in a bioassay. Ultimately, the “hot fractions” are analyzed with mass spectrometry to identify the responsible compounds. EDA studies often fail to identify the responsible toxic compounds, because, even after repeated fractionation, biologically active fractions remain too complex for chemical identification. A second problem is that current EDA studies are labor-intensive and as such not suitable for routine monitoring. This project aims to develop a rapid and sensitive platform for high-resolution identification of toxic compounds in the environment, mainly focusing at water. The project has three goals: 1. Development of a platform centralized around a spotter technology for nanofractionation onto high density microtiter plates (e.g. 1536 wells). The spotter technology provides high-resolution fractionation that maintains chromatographic separation efficiency. By integrating mammalian and other cellular bioassay responses and mass spectrometry, biological activity-to-identity correlation is greatly improved. 2. Miniaturization of mammalian cellular bioassays for high density microtiter well plates and high-resolution analysis formats for analysis of toxic activities in mixtures (e.g. water samples). 3. Applying this platform to water samples containing unidentified toxicants. Final goal is to provide a functional platform suitable for valorisation and subsequent distribution to end-users, e.g. surface water, wastewater and drinking water laboratories.

259 Relationships Between Antibiotics, Metals, and Antibiotic Resistance Genes in Sediments

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Antibiotic chemicals and antibiotic resistance genes enter then environment via wastewater effluents and runoff from agricultural operations. The relative importance of these two sources, however, is largely unknown. Because antibiotic chemicals may lead to development or retention of resistance genes by bacteria, the relationship between the concentrations of chemicals and genes requires exploration. Additionally, the genes that confer resistance to metal toxicity may also be important in antibiotic resistance. In this work, concentrations of 20 antibiotics (using liquid chromatography tandem mass spectrometry, 14 metals (using inductively coupled plasma – mass spectrometry), and 45 metal, antibiotic, and antibiotic-resistance associated genes (using a multiplex, microfluidic quantitative polymerase chain reaction method) were measured in 13 sediment samples from the Minnesota and Mississippi Rivers as well as along a transect in a wastewater effluent-impacted lake. Nine of the antibiotics were detected in the rivers and 13 were detected in the lake. Results indicate that the surrounding land use and proximity to wastewater treatment plants are important factors in the number and concentrations of antibiotics detected. Sixteen different resistance genes were detected in the samples. Correlations between antibiotic chemical concentrations, metal concentrations, and resistance genes occur over short (< 5 km) spatial scales within the lake system but are not apparent in the samples collected from the rivers.

260 Rapid Spatially Explicit Life Cycle Risk Assessment of Chemicals in Consumer Products - Case Study of Methylene Chloride in Paint Strippers

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The pace at which a life cycle assessment (LCA) is conducted for a chemical, is far slower than that of new chemicals being introduced into the market. Therefore, a tool to rapidly estimate the life cycle impacts of a chemical is needed to close the gap. The Chemical Life Cycle Collaborative (CLiCC) is a tool developed to enable a rapid assessment of the life cycle impacts of a chemical with little information required upfront. The CLiCC tool contains a comprehensive collection of experimental databases and predictive models for chemical properties, including human and ecological toxicity, and a spatially explicit dynamic risk assessment of chemicals with a set of models that estimate chemical releases, fate and transport, and multiple routes of exposure scenarios. The CLiCC tool utilizes best available data as much as possible. However, when facing data gaps, the predictive tools serve to generate estimates, which are transparently documented with their associated uncertainty estimate. In this presentation, we present a case study using methylene chloride (dichloromethane or DCM) used in paint strippers, to illustrate the workflow of the CLiCC tool. Due to its wide range of applications in high amounts and its potential carcinogenic properties, DCM was selected as a chemical of concern by the California Dept. of Toxic Substances Control under the Safe Consumer Products regulations. We compare results based on the USEPA's Risk Assessment and the CLiCC framework. Both assessments concluded that DCM may pose non-cancer risks to consumers. However, the amount of resources and data needed for running the CLiCC tool is much smaller. In addition, CLiCC can provide estimations of life cycle inventory (LCI) and characterized results using two different approaches, as well as a comprehensive uncertainty analysis. We demonstrate how the uncertainty analysis results of the CLiCC tool can be utilized as a way to prioritize additional data collection and modeling efforts to further refine the results.

261 Contaminants of Emerging Concern During De Facto Water Reuse

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The drinking water and wastewater cycles are integrally linked. Chemicals that are present in household wastewater may be sufficiently mobile and persistent to survive on-site or municipal wastewater treatment and post-discharge environmental processes. Such compounds have the potential to reach surface and ground waters which can be the sources of drinking water. The US Environmental Protection Agency (USEPA) and US Geological Survey (USGS) are collaborating on a project examining the sources, fates, and potential effects of contaminants of emerging concern (CECs) during de facto water reuse. The sampling design follows a surface water flowpath by collecting water samples from just above a wastewater treatment plant outfall and downstream to a drinking water treatment plant intake and through to a finished water sample. The study utilizes an integrated approach that includes a comprehensive analysis of specific chemicals (e.g. pharmaceuticals, perfluorinated chemicals, hormones, etc.), environmental diagnostics to identify non-target, unknown chemicals, in vitro bioassays (e.g. estrogenicity, androgenicity, genotoxicity, toxicity in metabolizing cells), rapid whole organisms screens (e.g. Microtox) to assess cumulative bioactivity, and in vivo tests to address specific exposure and response endpoints. A rigorous quality assurance/quality control protocol design is consistently applied from field to laboratory to ensure comparability of results from different techniques. This consistent, integrated approach combines the strength of each technique and is ideal for CEC related research in which traditional environmental and toxicity endpoints are not adequate for fully understanding potential effects to human health and the environment from chemical exposures. This presentation will provide an overview of the study, discussing the project design and preliminary results from the analysis of grab samples for organic and inorganic chemicals.

262 Characterization of Unknown N-nitrosamines and Their Precursors in Wastewater

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N-nitrosamines are probable human carcinogens. Arisen from upstream processes such as wastewater treatment, N-nitrosamines and their precursors have been detected in drinking water distribution systems and have drawn increasing concern. The USEPA targeted seven volatile N-nitrosamine species and developed detection methods. However, due to the complex matrix of municipal wastewater and the transformation in aquatic environment, these targeted N-nitrosamines only represents a small fraction (< 10%) of the total N-nitrosamine (TONO) pool, and limited information exists concerning the toxicity, sources and environmental fate of so far unknown N-nitrosamines and their precursors during wastewater treatment. To address this gap, we combine chemiluminescence detection and high-resolution mass spectrometry (HRMS) to characterize the fate and composition of wastewater-derived TONO and their precursors. We applied a previously developed chemiluminescence method to study the variation of concentrations of TONO across the treatment trains at six municipal WWTPs in New York. Out of the six WWTPs investigated, five exhibited higher TONO concentrations in the effluent prior to the final disinfection than those in the plant influent. Aerobic biological treatment (e.g., activated sludge or aerated filters) resulted in elevated TONO levels to various degrees, with the highest concentration reaching up to ~32,000 ng/L as NDMA. Final disinfection with ultraviolet light or sodium hypochlorite reduced TONO levels in most WWTPs. In most wastewater samples, N-nitrosamines concentrations increased 1-2 orders of magnitudes after chloramination formation potential tests, emphasizing the importance of TONO precursors. Our ongoing work is focused on screening and quantification of targeted volatile and nonvolatile N-nitrosamines and their precursors, with the goal to identify their relative importance in the TONO pool.

263 Mass Spectrometry Based Detection of Vitellogenin Peptides Across Fish Species for Assessing Exposure to Estrogenic Compounds in Aquatic Environments

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An increasing number of synthetic chemicals and natural hormones in the environment are being found to cause endocrine disruption in fish and wildlife. One of the most effective ways to evaluate the presence of endocrine disrupting chemicals (EDCs) in the aquatic environment is to measure the induction of vitellogenin (VTG) protein in male fish that inhabit the system. At present, measurement of VTG is generally performed using enzyme-linked immunosorbent assay (ELISA) or Southern blot, both of which rely on antibodies raised against VTG that are specific to the fish used to generate the VTG. In the present study, we developed a liquid chromatography with tandem mass spectrometry (LC-MS/MS) method to measure common peptides found in VTG from different fish species, as an alternative to the commonly used but species-specific ELISA and Southern blot techniques. Two experiments were designed. First, common peptide candidates resulting from the trypsin enzymatic hydrolysis of VTG from three fish species were identified using high-resolution mass spectrometry. This process involved protein isolation by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), in-gel enzyme digestion, and peptide identification using a quadrupole time-of-flight mass spectrometer (Q-TOF/MS). Second, to allow detection of low concentrations of the target peptides, LC-MS/MS analysis of the identified common peptides (ALHPELR and FIELIQLLR) were performed under multiple reaction monitoring (MRM); these peptides served as surrogates for VTG in samples from real fish that had been exposed to estrogenic compounds. The transitions *m/z* 418->288, *m/z* 418->326, and *m/z* 418->514 were used to monitor ALHPELR. The transitions *m/z* 573->642, *m/z* 573->885, and *m/z* 573->756 were used to monitor FIELIQLLR. Functional validation of these signature peptides was performed by comparing their amounts in trypsin-digested serum of female fish (positive control), estrogen-exposed male fish (test sample), and unexposed male fish (negative control) in three fish species, namely *Pimephales promelas, Micropterus salmoides,* and *Ameiurus nebulosus*. Results from our study demonstrated the potential of LC-MS/MS as a generic method for measuring common VTG peptides from the serum of fish species that have been exposed to EDCs. This generic method will allow exposure assessment of multiple fish species to EDCs as an alternative to ELISA, which uses species-specific antibodies that typically only measure VTG in single fish species.

264 Detection of physiological activities of antidepressant pharmaceuticals in wastewater by fluorescence-based in vitro assay

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Over recent years, growing numbers of human pharmaceuticals have been detected in effluents of wastewater treatment plants (WWTPs) and river water. Concern about their potential risks to aquatic species has been raised because they are designed to be biologically active. One of most concerned pharmaceuticals are antidepressants. For example, selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine and sertraline could alter the behaviour of fish in vivo testing. Wide variety of antidepressants such as SSRIs, serotonin-norepinephrine reuptake inhibitors (SNRIs), and dopamine reuptake inhibitors (DRIs) are now on the market worldwide. It is possible to measure the concentrations of selected antidepressants by chemical analysis, but such concentrations do not indicate the physiological activity of the antidepressants in waters. For example, even if the concentration of each substance is low, through additivity compounds might produce a strong enough physiological activity to harm aquatic organisms. To determine whether antidepressants in aquatic environments alter the behaviour of aquatic organisms, we must know the extent to which such organisms may be exposed to antidepressants as determined by the inhibition of monoamine transporters. In this study, we measured the physiological activity of antidepressants in WWTP effluents for the first time using the fluorescence-based in vitro assay (namely called antidepressant assay). We utilized the fluorescence substrate for monoamine transporters (serotonin (SERT), norepinephrine (NET), and dopamine (DAT) transporters). At first, inhibitory activity of antidepressants (e.g. IC50) were determined in HEK293 cells transiently-transfected with human recombinant SERT, NET, or DAT. Then, we applied the antidepressant assay to secondary effluent collected at the WWTP in Japan, and succeeded to detect the functional inhibition of SERT. Importantly, strong inhibition was detected in SERT-expressing cells, but neither in NET-expressing nor in DAT-expressing cells. These results indicate that detected inhibitory activity come from specifically SSRIs in wastewaters, neither from the adsorption of fluorescence substrate to large organics molecules nor interfering fluorescence in wastewater. Activities detected in waters could be quantified as antidepressant-equivalent quantities (e.g. sertraline-EQ/L). Fluorescence-based in vitro assay is very useful to measure the physiological activity of antidepressants in waters.

Ecoxicoxicity of Per- and Polyfluoroalkyl Substances (PFASs)

265 Ecological Risk Assessment of Aquatic PFAS Exposure at an Air Force Base: Site characterization and preliminary risk assessment

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Perfluoroalkyl and Polyfluoroalkyl substances (PFAS) are emerging contaminants of concern. Their historical use in a wide variety of consumer products and fire-fighting foam formulations has led to concerns regarding environmental contamination and potential adverse effects in humans and wildlife. We sought to better understand the distribution and potential risk of PFAS in and around Barksdale Air Force Base, LA (BAFB), where fire-fighting foams had been used historically. We sampled sediment, water, and aquatic biota from bayous near two former fire-training areas and several reference locations within BAFB. We focused our analysis on six specific PFASs and we found perfluorooctane sulfonate (PFOS) and perfluorohexane sulfonate (PFHxS) at the highest frequency and magnitude of detection but focused our risk assessment on PFOS because of a lack of PFHxS toxicity data. These PFASs were also commonly detected in fish tissues, sometimes at concentrations (in muscle) that exceed some provisional health advisory levels. We conducted a series of ecotoxicity studies on aquatic animal species to better characterize potential toxicity of PFOS and used these data to build a species sensitivity distribution. We then generated log-normal probability distributions for PFOS exposure at several different locations within the sample bayou at BAFB and determined a probability that several toxicity benchmarks for aquatic species were exceeded. The three toxicity benchmarks we used were similar in magnitude (0.5 – 5 ppb) but the probability of exceedance varied from 0.04 to 0.49 depending on the sample location and the benchmark. This analysis suggests that available toxicity benchmarks for aquatic species could be exceeded by surface water concentrations of PFOS at BAFB which may lead to adverse effects. We also observed a lower fish condition index in largemouth bass associated with higher tissue PFOS concentrations providing some empirical evidence for a potential negative effect of PFOS in this system. That said, there are considerable uncertainties with the analysis including the possibility of non-PFAS contaminants, poor temporal resolution in surface water PFAS concentrations and the toxicity of PFHxS and PFAS mixtures. Nonetheless, this assessment suggests that additional research and assessments for ecological effect of PFASs are warranted.

266 A spatially-explicit perfluorooctane sulfonate (PFOS) uptake and depuration model for fish using data from the laboratory and field

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Perfluoroalkyl substances (PFASs) such as perfluorooctane sulfonate (PFOS) were used for their flame-retardant properties until they were phased out by the manufacturer in the early 2000's. However, these chemicals continue to receive considerable interest within the scientific community due to their persistence in the environment (PFOS has a 40-year half-life in aquatic systems) and potential to cause adverse effects over a chronic exposure duration. Importantly, these adverse effects manifest in both the ecological and human receptors due to consumption of contaminated food items. Risk assessments of contaminated sites can be improved by the inclusion of models that predict organismal burdens after exposure, however, considerable uncertainties remain regarding the extent that PFOS accumulates and depurates in fish. To begin to address these issues, we developed three one-compartment bioconcentration models using a kinetic bioconcentration factor for uptake and depuration over time for PFOS in fish, representing uptake into muscle, blood, and liver. These models were then compared to data obtained from: (1) peer-reviewed sources, (2) a study conducted in the laboratory using *Gambusia affinis*, and (3) from an historically contaminated field site, Barksdale Air Force Base (BAFB), in Louisiana. We then further refined the model by adding a spatially-explicit component using the freeware program NetLogo, to better account for exposure variability such as would be observed in the field. The model includes a number of useful features, including the ability to modify the size of the home range of the fish and the total number, size, and mean and standard deviation of concentration of reaches of a water body. This model provides a range of final tissue concentrations instead of a single value and therefore may provide a better approximation of exposures in the environment. We tested this model with field-derived data and the model closely approximated observed concentrations in many fish species collected at BAFB when parameterized with bioconcentration kinetics factors derived from *Oncorhynchus mykiss*. As developed, and with the potential to include further refinements as research continues to be published on PFOS accumulation in fish, the spatially-explicit model can provide risk assessors with a useful tool in both environmental and human health risk assessments.

267 Strategies to prioritize PFASs mixture identification for ecotoxicity testing and risk assessment

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There is growing concern regarding the health and environmental effects of per- and polyfluoroalkyl substances (PFASs). These compounds have been measured in a wide variety of human, wildlife, and environmental samples. Of these compounds, perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) have received the most research attention, are the most frequently detected, and are often considered the majority compound of PFASs present. However, these two PFASs seldom occur singly and are not exclusively dominant components of PFAS mixtures detected in environmental samples. Mixtures of PFASs often contain constituents such as perfluorohexane sulfonate (PFHxS), perfluorohexanoic acid (PFHxA), and perfluorobutane sulfonic acid (PFBS), for instance. In some cases, these less studied compounds make up majority constituents in environmental surface water samples. This is of particular importance as the lack of toxicity data on minority constituents and even less data on mixture toxicity presents a roadblock to assessing risks of PFASs. Hence, an important research goal is to identify relevant PFAS mixtures for toxicity testing and risk assessment. In order to identify PFAS mixture formulas that are environmentally relevant and broadly applicable, we applied a prioritization scheme to data on PFAS surface water concentrations from several locations. Our prioritization scheme clusters sites and chemicals by the quality of data available, measured concentrations, and variability in constituent make-up. The results of our prioritization efforts provide useful output for identifying (1) the most relevant PFAS chemicals and mixtures and (2) particular sites or PFAS chemicals that likely warrant immediate management or research attention. Additionally, analysis of several data sets indicates that core components of common PFAS mixtures (e.g. PFHxS and PFBS) are seldom-studied compounds and that some commonly studied compounds (e.g. PFOA) are not universally major constituents. In summary, we have developed methods for PFAS prioritization that aids in defining a broadly environmentally relevant PFAS mixture. As PFASs in the environment are seldom solitary, this information and methodology could be used to guide future research that can reduce uncertainties regarding the environmental and health effects of PFASs.

268 Selection of Aquatic Toxicity Data for Species Sensitivity Distributions for PFOS and the Effect on Aquatic Life Protection Criteria

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Information on the potential adverse effects to aquatic species from exposure to perfluorooctane sulfonate (PFOS) has grown significantly in recent decades, as has regulatory concern and the need for decision making criteria for the protection of aquatic life. While the regulatory focus has been on drinking water guidelines and standards, some agencies have promulgated criteria for the protection of aquatic life for PFOS. Recently, the Australian Department of the Environment and Energy (DoEE) released draft guidelines for PFOS that included a freshwater criterion of 0.00023 µg/L, which is hypothesized to be protective of 99% of freshwater species (99% Species Protection Value). This value is considerably lower than the values put forth by other regulatory agencies using similar Species Sensitivity Distribution (SSD) approaches. Following careful review of the toxicity data included in the DoEE SSD, it was determined that a single toxicity value for zebrafish (*Dario rerio*) was exerting a strong influence over the entire SSD and the 99%, 95% and 80% species protection values, as it was more than 10X less than the next lowest toxicity value. As a result, the 99% Species Protection Value was highly uncertain, with the 95% Confidence Interval (95% CI) on the estimate ranging seven orders of magnitude (i.e., < 0.000001 to 3.1 µg/L). The source of the toxicity value (Keiter et al. 2012) was critically reviewed to determine if appropriate, ecologically relevant toxicity data were selected. The No Observed Effects Concentration (NOEC) value for zebrafish included in the SSD based on reduced growth was 0.294 µg/L. However, critical flaws in the derivation and selection of the NOEC were identified. Following review of the magnitude of effects and applying an ecological significance benchmark of > 20% adverse effect – a benchmark generally applied in ecotoxicology as indicative of measurable effects to a population outside of natural variability – a more appropriate NOEC of 106 µg/L was identified and included in the SSD. The 99%, 95% and 80% Species Protection Criteria (95% CI) were re-calculated using the revised SSD, resulting in criteria of 1.2 (< 0.01 – 5.4), 5.7 (< 0.01 – 24), and 40 (28 – 465) µg/L, respectively. These revised values demonstrate reduced uncertainty and are in better agreement with other decision-making criteria produced using SSDs, as well as the empirical values upon which they are derived.

269 Acute Toxicity of Perfluorinated Chemicals in Japanese Quail (Coturnix japonica)

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Aqueous film forming foam (AFFF) has been used by the Department of Defense for over 40 years for fire-training and emergency response activities. As a result of these activities, ground water, surface water and biota in the vicinity of relevant military installations have become contaminated with perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and other poly- and perfluoroalkyl substances (PFASs). As part of an effort to develop avian ecotoxicity information for compounds associated with AFFF, the acute toxicity of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) separately and in combination in an avian species recognized as a surrogate for wild avian species, the Japanese quail (*Coturnix japonica*), was determined. Ten-day-old Japanese quail were administered PFOS at nominal dietary concentrations of 0, 70, 141, 281, 562, 843, 1125, 1687, or 2250 µg/g feed or PFOA at 0, 200, 350, 500, 625, 750, 1000, 1250, or 1500 µg/g feed for five days and then fed untreated feed for an additional 18 days. Half of the birds were euthanized on day 8 of the trial and sampled for blood and liver. The remaining birds were euthanized at the end of the 23-day trial and sampled as above. Liver and pooled serum samples were analyzed for PFOS or PFOA. Based on the average daily intake of PFOS calculated over the five-day exposure period, the LD50 was 56.1 (49.1-64.0) µg PFOS/g bw/d, equivalent to a cumulative dose of 281 µg PFOS/g bw. The five-day LC50 was 409 (310-542) µg PFOS/g feed. At the end of the 23-day trial, survivability was 100% in the 0, 70 and 141 µg/g feed groups, 15% in the 281 µg/g feed group and 0% in the 562, 843, 1125, 1687 and 2250 µg/g feed groups. By day 5 of the trial, body weights of PFOS-treated birds were significantly less compared to controls, which was attributable to a corresponding decrease in feed intake. After provision of clean feed on day 6, average body weights of surviving birds increased, although only average body weight of birds in the 70 µg PFOS/g feed group was comparable to the control group by the end of the trial. Average absolute liver weights were significantly less in all treated groups compared to controls at Day 8, but average liver weights expressed as a percent of body weight were not. There were no significant differences in absolute or relative liver weights at the end of the trial.

270 Prioritizing Data Needs For Assessing the Ecological Risks of PFASs in Habitats Impacted by Aqueous Film Forming Foam Releases

J.M. Conder, E. Larson, J. Arblaster, Geosyntec Consultants

Perfluoroalkyl and Polyfluoroalkyl Substances (PFASs) have been widely used in numerous industrial and commercial applications since the 1950s, including aqueous film forming foams (AFFFs) used for fire suppression at airports, firefighting training facilities, and other industrial locations. PFASs such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are now routinely-detected in a wide variety of environmental media impacted by AFFF, and have prompted regulatory focus on exposures and risks. Many AFFF sites host ecological habitat or, due to the offsite transport potential for PFASs (especially for aquatic transport routes), may affect nearby or downgradient habitats. Unfortunately, there is little guidance on developing Conceptual Site Models (CSMs) and investigation plans to evaluate the ecological risks of PFASs at AFFF sites. This presentation will highlight key ecological risk considerations for PFAS at AFFF sites, based on reviews of PFAS fate and toxicology information, as well as ecological exposure modeling of PFAS exposures for wildlife at AFFF sites. Our work indicates that PFOS is likely to be a key PFAS of concern at many sites, particularly for avian wildlife. For example, PFOS comprised approximately 80% of the exposure of perfluoroalkyl acids to avian species at five AFFF sites, likely due to its prevalence in many historical AFFF formulations, ability to bioaccumulate in terrestrial and aquatic food webs, and partitioning to soils and sediments. However, exposures to other PFASs, especially perfluorohexane sulfonate (PFHxS) and perfluorodecane sulfonate (PFDS) may also be important, and suggest that avian toxicity testing should be prioritized for these particular PFASs. Model and review results also highlighted several practical steps for site-specific investigations for supporting ecological risk assessments, including: 1) focusing investigations on aquatic systems affected or downgradient of AFFF discharge areas; 2) screening measurements of PFASs in key abiotic environmental compartments (soils, sediments, and surface water); 3) measurements of PFAS in invertebrates and fish; and 4) measurements of organic carbon contents in soils and sediments to aid with modeling fate and availability. Additional considerations for research needs and site-specific ecological risk management approaches will be presented, with the hopes of optimizing ecological risk-based decision making at AFFF sites.

271 Examination of the Sub-lethal Effects of Four Per/polyfluoroalkyl Acids (PFAAs) on Two Species of US-native Amphibians

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Per/polyfluoroalkyl acids (PFAAs) are an emerging group of organic pollutants. Widespread use along with volatility of some PFAAs has led to global distribution in soils and surface waters. The chemical stability of these compounds assures persistence in the environment, with unknown effects to much of the chronically-exposed biota. Many toxicology studies implicate PFAAs in disruption of thyroid hormone, which is also known to play a major role in control of metamorphosis in amphibians. Thus, amphibians represent a class of organisms that may be susceptible to toxicity following PFAA exposure. In this study, we examined the effects of aqueous exposure of four different PFAAs at environmentally-relevant doses (ranging from 10-1000 ppb) on larvae of two amphibian species: eastern tiger salamanders (*Ambystoma tigrinum*) and American toads (*Anaxyrus americanus*). The OECD amphibian metamorphosis assay (Test 231) was used as a guideline for these experiments. Larval salamanders were exposed for 10 days of accumulation followed by a 22-day chronic exposure. Measurements of length and mass were compared between control and treatment groups. Toad tadpoles were exposed from Gosner stage 26-28 through climax of metamorphosis, defined as Gosner stage 43-44. Length, mass, and time to metamorphosis were compared between control and treatment groups. In addition, histopathology of the thyroid glands of control and treatment animals from both species were examined at the conclusion of each experiment. Alterations in morphology of thyroid follicular cells, colloid deposition, and overall architecture were noted. This study presents the first in-depth look at potential effects of PFAAs on growth, development, and endocrine disruption at environmentally-relevant doses on US-native amphibians.

272 Toxicological Response of Chironomus tentans to Six Perfluoroalkyl Compounds

C.J. McCarthy, CH2M / Environmental Services; M. Stanaway, CH2M / Applied Sciences Laboratory; C. Salice, Towson University / Environmental Science & Studies Program; T. Wright, USEPA / Office of Pollution Prevention and Toxics

A multi-facetted bioassay study was conducted on six different perfluoroalkyl substances (PFAS) with the intent of obtaining data to evaluate relative or proportional toxicity between the substances. The six substances were selected off of the EPA's third Unregulated Contaminant Monitoring Rule (UCMR3) list. Two of the substances, perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), are well studied. The other four substances (Perfluorononanoic Acid [PFNA], Perfluorobutanesulfonic Acid [PFBS], Perfluorohexanesulfonic Acid [PFHxS], and Perfluoroheptanoic Acid [PFHpA]) are commonly detected in groundwater around the US, but far less is known about their toxicological potential. 10-day acute range finding tests and 20-day chronic definitive renewal bioassays were run on all six compounds. Exposure doses were established above and below environmentally relevant concentrations. Measurement endpoints included: percent survival; growth; time to emergence; number of emergent adults; and number of larvae and pupae at test termination. Results from the 10-day acute survival tests were used to inform dosing for the 20-day chronic growth tests. Dose response curve fitting was performed for each compound. Additional statistical analysis was performed to assess the relationship between the more toxic PFOS and the other compounds. A second round of chronic testing was performed to investigate potential additive and synergistic toxicity. Organisms were exposed to mixtures containing differing proportions of the 6 compounds, based upon ratios of detected concentrations in field collected data. Results are in concert with previous work showing PFOS to be the most toxic PFAS and additive toxicity with PFHxS. Additive toxicity with other PFAS appears less clear and needs further investigation. Results of this work will be considered in conjunction with other ongoing work testing the same compounds and mixtures to look for patterns of similarity among differing classes of organisms.

Whole Effluent Toxicity Testing: A Science Evolving – Perspectives, Alternatives, and Regulatory Limitations

273 Contaminant effects in Gambusia affinis from two impacted rivers by treated municipal effluents

P. Ngwenya, Philander Smith College / Biology

Freshwater rivers and streams are common repositories for the discharge of large volumes of industrial and domestic waste, mainly through wastewater treatment plant (WWTP) effluent. One common group of chemical pollutants present in WWTP effluent are endocrine disrupting chemicals

(EDC's). EDC's can induce morphological and reproductive changes in aquatic organisms. This aim of this study is to compare morphology and reproductive development, and determine whether populations exhibit changes in sex ratio in mosquitofish (*Gambusia affinis)* collected from an urban and a rural river impacted by treated municipal effluents. Rivers will be sampled at the discharge point of effluent release and 250m upstream and 250m downstream of the release point. Data is still being collected, therefore, results will be presented at the meeting.

274 Development of the Fish Gill Cell Line (RTgill-W1) to Evaluate Whole Effluent Toxicity in-vitro and compare with effects in-vivo

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The clean water act established environmental regulations for wastewater facilities that discharge effluents into native surface waters by requiring them to perform Whole Effluent Toxicity (WET) testing. WET tests are biomonitoring assays that use live laboratory organisms to evaluate the effects of potential toxicants to native aquatic populations. Current WET test methods are rather laborious, not very cost effective, and lack detail to identify the toxicant mode of action. Moreover, the use of live animal testing has become a growing ethical concern. The RTgill-W1 cell line is a well-established gill epithelial model which has been used extensively as an alternative to whole animals testing in aquatic toxicology studies. The fish gill is an important multifunctional organ constantly bathed in water and the main site of interaction with contaminants dissolved in water. Utilization of cultured cell lines offers a rapid, high throughput and easy to use approach which allows simultaneous measurement of multiple toxicity endpoints in biomonitoring assays. However, exposure conditions require careful consideration, as cell lines cannot be exposed directly to low osmolarity water samples. In this study, we have conducted a series of experiments to evaluate RTgill-W1 as an alternative *in-vitro* method for WET testing. To evaluate osmolarity tolerance of RTgill-W1, cells were exposed to synthetic media of varying osmolarity. Cell viability was evaluated by indicator dyes that measure cell metabolism and membrane integrity. Dose–response curves were generated over a period of 24h to calculate effect concentrations (EC50s). EC50s of 14 single toxicants commonly found in effluent samples will be correlated to lethality in live organisms (LC50) from literature data. RTgill-W1 optimization of exposure medium has shown that cells can tolerate a range of osmolality between 100 and 500 mOsm/Kg. Optimization for WET testing will be evaluated in different exposure media (i.e. hyposmotic, isosmotic, and hyperosmotic conditions). This procedure has the potential not only to be used for WET testing purposes, but also as a complement to current toxicity identification and reduction evaluation strategies for effluent testing. Furthermore, this concept serves to facilitate the use of in vitro methods as an alternative to current *in-vivo* methods which will drastically reduce the amount of fish needed to perform WET tests.

275 Whole effluent toxicity testing: Is there a non-vertebrate approach?

L.A. Kristofco, Baylor University / Environmental Science; S.A. Hughes, Shell Health Americas

Royal Dutch Shell (Shell) employs animal testing for the hazard assessment of chemicals and products, research and development testing, and effluent discharge compliance. 84% of ~102,000 of the vertebrate organisms used in 2015 were fish employed in whole effluent toxicity (WET) tests for regulatory compliance applications. Of this, 64 % of Shell's global fish use in 2015 was from its North American operations. In an effort to decrease the dependence on vertebrate testing for compliance, Shell is proactively seeking non-vertebrate (alternative) testing approaches to trial as potential replacements for traditional vertebrate methods currently used for WET testing. To identify potential alternative methods, an extensive literature review was undertaken to identify and prioritize potential non-vertebrate alternative methods. This selection process identified trends in the bioassays applied regionally and elucidated alternative approaches that harmonize global testing requirements. The toxicity testing methods evaluated were selected from international standard organizations and WET methods previously used by Shell globally. These were further refined based on their applicability to effluent testing in the North American compliance framework. Priority was given to those methods that were available commercially in North America, were freshwater, and included apical endpoints in methods similar in duration and style to current compliance testing methodologies. Additional priority was given to methods which had the greatest similarity to fish. An initial list of 64 standard protocols from agencies from around the world were compiled and refined to a list of 7 that reflected 5 testing frameworks for further performance testing. The advantages and challenges associated with each method were investigated. An overview of Shell's approach to eliminating vertebrate testing will be presented, and will include a discussion of a combination of alternative in vitro and in vivo techniques. It may be that no single method or test by itself is sufficient to replace vertebrate testing, but using a weight of evidence approach could help to reduce vertebrate testing.

276 Ceriodaphnia dubia Chronic Toxicity Test Variability: Is There a Need for Method Improvement?

S.L. Clark, B.C. Jorgenson, A.M. Briden, R. Ogle, J.S. Cotsifas, Pacific EcoRisk

Since 1985, USEPA has released four editions of the testing manual for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms, with the last version of the manual being released in 2002. Each revision to the manual has included improvements to the methods, including culturing, test conditions, and quality control, based on increased experience of laboratories performing the testing, while still providing laboratories with flexibility for a variety of test method elements. During the 1980s and 1990s when the methods were undergoing revisions, there was a collaborative atmosphere among laboratories that were performing the methods, including the formation of the Northern California and Southern California Toxicity Assessment Groups (NCTAG and SCTAG) where laboratories shared their experiences with the methods. However, there has been little collaboration among laboratories working on these methods since then, even though there is a growing body of data that suggests that there is further need for method improvements. For example, several recent publications have indicated that the results of tests using the chronic *Ceriodaphnia dubia* survival and reproduction test can vary significantly among laboratories. The magnitude of this issue was further demonstrated in an inter-laboratory comparison study coordinated by the Southern California Coastal Water Research Program in 2015/16, in which the chronic *Ceriodaphnia dubia* test was identified as the most variable method in their study. The result of such variable test results is diminished confidence of the regulated community that is required to use this method for their compliance determinations. Where the method is required for compliance determination, such variability results in ambiguity for regulatory staff. This has been further exacerbated by parties that compare results from two different laboratories without the use of referee laboratories to resolve how to address results should they differ between labs. In this poster, we provide chronic *Ceriodaphnia dubia* test data from multiple inter-laboratory studies, propose potential drivers for variability in the outcomes of such studies, and encourage greater dialogue and studies among experts in the field so as to increase the confidence of both the regulated community and regulatory agencies that require the method for compliance determinations.

277 Comparative Analysis of Side-by-Side Chronic Ceriodaphnia dubia and Daphnia magna Four-Day Survival and Growth Test Methods in Various Waters

N. Love, S. Skigen-Caird, GEI Consultants, Inc.; D. McCausland, GEI Consultants, Inc. / Ecology

For the last three years, GEI Consultants, Inc. (GEI) has been performing the *Daphnia magna* four-day survival and growth test method procedure presented in 2009 by James Lazorchak, Mark Smith, and Herman Haring. In that timeframe, we have completed approximately 70 chronic

Ceriodaphnia dubia tests concurrently with the four-day *D. magna* test. These tests have been conducted on effluents from three active mine sites, one domestic wastewater treatment plant, and a legacy mine site now in remediation, as well as surface water samples from two instream locations. All samples were collected from sites in Colorado. While the four-day *D. magna* method has not been approved by the United States Environmental Protection Agency, we have begun to evaluate concurrent chronic *C. dubia* and four-day *D. magna* tests to determine whether the method could potentially isolate the total dissolved solids (TDS) component while still providing an estimation of true toxicity. GEI's database was evaluated to identify potential differences in species sensitivity to different effluent components. These datasets were also evaluated to detect any trends in the results between industries; all findings will be presented.

278 Toxicity Assessments for NPDES Compliance: Traditional TSD methods versus the TST approach

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Compliance parameters used for NPDES permits typically employ physical, chemical, and ecotoxicological methods as a suite of tests to determine the likelihood of an effluent discharge as having an adverse impact to the receiving waterbody. Traditionally, acute and chronic toxicity testing have been used for assessing whole effluent toxicity (WET) to fishes, invertebrates, and plant surrogate test organisms in laboratory exposures. The Technical Support Document for Water Quality-based Toxics Control (TSD) recommends multiple concentration series expsoures (five concentratiosn and a control) to determine the acute and/or chronic toxicity of the effluent (USEPA 1991). Over the years, the USEPA has updated and revised the specific toxicity test methods (e.g., USEPA 2002a-for acute and USEPA 2002b-for chronic testing) such as allowing regression analysis as a tool for assessing toxicity in addition to hypothesis testing techniques. However, the test methodologies consistently recommended the use of mutiple test concentrations for assessing both acute and chronic effluent toxicity. In 2010, the EPA released a set of technical documents that presented an alternative statistical method called the Test for Significant Toxicity (TST), which employed a two treatment test (an effluent concentration at the instream waste concentration and a control) as a pass-fail compliance test using hypothesis testing techniques (USEPA 2010a, b). The TST approach offered another statistical option to analyses of valid WET test data. The documents further stated that EPA Regions and States could still use the EPA's traditional TSD approaches. The TST strategy also turned the question of compliance from the traditional expectation of an effluent being non-toxic until determined to be toxic, as compared to the TST approach which assumes the effluent is toxic until it is determined that it is non-toxic. This paper will discuss the advantages and disadvantages of the alternative TST strategy as compared to the traditional TSD strategies with regards to performance, interpretation, and compliance decisions and the potential implications to individual NPDES permittees.

279 The Value and Challenges of WET as a Compliance Tool

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Whole effluent toxicity (WET) testing is one of the three quality-based toxics control approaches developed and used to monitor and protect aquatic ecosystems from toxic discharges in toxic amounts. WET and other toxicity tests are unique in that they provide an overall assessment of the myriad of possible chemicals present in the aqueous sample or effluent, unlike a chemical-specific approach that focuses on one chemical at a time, or field evaluations that directly measure potential impacts in the receiving water sites but can be cost prohibitive. This holistic approach is one of the major advantages of WET as the organisms or 'detectors' are exposed to any potential physical, chemical, and/or biological stressors in the aqueous sample. However, WET can and often does get a bad reputation with concerns about appropriate representativeness, quality assurance / quality control, appropriate sensitivity of the tests species, and overall usability of the data generated. While there are protective measures in place to help ensure the data quality objectives are met, it ultimately comes down to the specific laboratory conducting the work and how confident stake holders are in the work that is being generated. The heterogeneity and sometimes inconsistency in accreditation programs / review standards for WET laboratories can provide challenges for an 'apples to apples' comparison among even accredited WET laboratories. This paper will discuss some of the challenges facing WET and some suggestions for dealing with those instances to hopefully improve the quality and usefulness of WET data.

Fate and Effects of Metals – Mechanisms of Toxicity

280 Metabolic and cardiovascular effects of dietary selenomethionine exposure in adult rainbow trout (Oncorhynchus mykiss)

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Selenium (Se) is an essential micronutrient involved in important metabolic functions for all vertebrate species. As Se is reported to have a narrow margin between deficiency and toxicity, there is growing concern surrounding the adverse effects of elevated Se exposure caused by anthropogenic activities. Oviparous vertebrate species, especially fish, are highly susceptible to elevated dietary Se exposure. Recent studies have reported that elevated dietary exposure of fish to selenomethionine (Se-Met), the primary form of Se in the diet, can drastically alter cardiovascular function, metabolic capacity, energy homeostasis, swimming performance and cause a greater incidence of early life stage deformities and mortality. This study aims to further investigate mechanisms of Se-Met toxicity, particularly potential underlying cardiovascular implications of chronic exposure to environmentally relevant concentrations of dietary Se-Met in adult rainbow trout (*Oncorhynchus mykiss).* Adult rainbow trout were fed either control food or Se-Met spiked food (6.4, 15.8 or 48.8 µg Se/g, dry weight) for 60 days at 5% body weight per day. Following exposure, high resolution B-mode and Doppler ultrasound was used to characterize cardiac function. Chronic dietary exposure to Se-Met significantly increased stroke volume, cardiac output, and ejection fraction. Fish fed with Se-Met spiked food had elevated liver glycogen and triglyceride stores, suggesting impaired energy homeostasis and metabolic dysfunction. Expression of mRNA genes of interest was quantified by use of Real-Time Quantitative Polymerase Chain Reaction (qRT-PCR). Exposure to Se-Met significantly decreased mRNA expression of citrate synthase (CS) in liver and serpin family H member 1 (SERPINH1, a 47 kDa heat shock protein and chaperone in collagen processing) in heart, and increased abundance of sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) and a key cardiac remodeling enzyme matrix metalloproteinase 9 (MMP9) in the rainbow trout heart. Oxidative stress markers glutathione-S-transferase (GST), glutathione peroxidase 1A (GPX1A) and catalase (CAT) were all elevated in livers of Se-Met exposed trout. These effects may lead to further cardiovascular and metabolic complications. The results of this study suggest that chronic exposure to dietary Se-Met can alter both cellular and physiological responses, and such consequences could threaten fitness and survivability of adult fish.

281 Role of Chloride on the Toxicity, Uptake, and Bioavailability of Silver and Copper in a Rainbow Trout Gut Cell Line (RTgutGC)

M. Ibrahim, M. Minghetti, Oklahoma State University / Integrative Biology

Generally, it is believed that the ionic form of a metal is toxic while its complexation with ligands may reduce the toxicity. However, there is a growing evidence that metal complexes may become bioavailable and elicit toxicity. Metals form complexes when they react with organic and inorganic ligands. Among inorganic ligands, chloride (Cl) is a ubiquitous element and is present in freshwater at 0.1-1 mM, in different waste waters at a range of 2-20 mM, and in sea water at 0.5 M. Recent studies have shown that toxicity of copper (Cu) and silver (Ag) in fish are higher at low and high salinity or chloride concentrations, but lower at intermediate concentrations. Although the exact mechanism by which salinity influences toxicity of metals is not clear, formation of metal-chloride complexes may influence the toxicity. Thus, in this study, we investigated the effects of chloride concentrations in exposure media on the uptake, toxicity, and bioavailability of Cu and Ag using an *in-vitro* model of the rainbow trout gut, the RTgutGC cell line. Cells were exposed to synthetic media of well-defined composition with varying Cl concentrations (0-145 mM). Visual MINTEQ (a chemical equilibrium model) was used to determine Ag and Cu speciation in exposure media. Cellular toxicity and dose-response curves were evaluated using a viability assay based on three endpoints: metabolic activity, membrane, and lysozyme integrity. It was found that ionic Ag^{+} was dominant between 0 and 1 mM Cl, and negatively charged Ag complexes (mainly $AgCl_2^{-}$) were dominant from 10-145 mM Cl. Copper speciation remained stable across the chloride concentration gradients, mainly complexing to hydrogen phosphate(45%) or remained in ionic form, Cu^{2+} (40%). In our current studies, RTgutGC will be exposed to Ag and Cu at a non-toxic concentration in media of varying Cl concentrations to evaluate the effect of chloride on metal uptake and bioavailability measured by ICP-MS and Metallothionein mRNA levels, respectively. Currently, the Biotic Ligand Models (BLMs) do not consider metal complexes as toxic. But recent data show that metal complexes can be toxic and bioavailable. Therefore, understating the contributing factors to these processes could be used to improve the predicting power of models.

282 The influence of pH on zinc uptake by the aquatic insect Zapada

D.J. Cain, USGS / Water Resources Division; M.N. Croteau, USGS; C. Fuller, USGS / National Research Program Western Branch

The influence of pH on Zn influx as well as whole body concentrations of Ca, K, and Na was examined in an acid- and metal-tolerant aquatic insect, the stonefly *Zapada* spp., to better understand the underlying physiological mechanisms governing its metal tolerance. Test specimens were collected from a neutral pH (7.5) stream and acclimated to synthetic test water formulated to the major ion chemistry of the stream and adjusted to four pH levels (3.8, 5, 6.2, 7). Each pH treatment was spiked with varying amounts of ^{67}Zn to establish three concentrations of $^{67}Zn^{2+}$ ranging from 0.1 to 8.4 µM. Influx rates of ^{67}Zn following a 4-hour exposure displayed Michaelis-Menten type kinetics. Data were fit using a one-site binding saturation model. Decreasing pH decreased the maximum binding capacity, B_{max}, and had relatively little effect on the half-saturation constant, K_d, except at pH 3.8 where it appeared to increase to 5.2E-06 M from 1.3E-06 M at pH 7. The dissociation constant for H^{+} and the binding site complex, K_i, was estimated among treatments to be 1.1E-05 ± 9.6E-06. The respective binding site affinities for Zn^{2+} and H^{+} indicate the competitive capability of H^{+}. Generally, pH did not appear to perturb major cation homeostasis. No effect of pH on either Na or K whole body concentrations was detected, while a pH effect on Ca was ambiguous. Ca whole body concentrations varied 17% among pH treatments, with concentrations at pH 7 and 3.8 lower than those at pH 6.2 and 5. The findings suggest that the insect's tolerance to metal-enriched acidic water involves noncompetitive inhibition, rather than strictly direct competition between H^{+} and Zn^{2+} for an active binding site. Noncompetitive inhibition by H^{+} suggested in this study for an aquatic insect has also been reported for other metals in algae. Such effects need to be explicitly considered in bioaccumulation models to simulate metal uptake among waters with different acidity.

283 Understanding the biogeochemical controls of uranium bioavailability from the dissolved and dietary phases in a model invertebrate species

M.N. Croteau, USGS; C. Fuller, USGS / National Research Program Western Branch; D.J. Cain, USGS / Water Resources Division; K. Campbell, USGS

Growing worldwide demand for uranium (U) as an energy source has raised concerns about the human and ecological risks of U extraction and processing. Determining the environmental health effects of U requires, however, understanding the exposure pathways leading to bioaccumulation, and its potential risks to receptors. A great deal of information exists on the toxicity of U to freshwater organisms, but far less is known about the underlying mechanisms governing U bioaccumulation in invertebrates. Understanding these mechanisms is needed to better evaluate toxic effects. Here we investigated pathways of U exposure and biogeochemical controls on U bioavailability in a model species, the freshwater snail *Lymnaea stagnalis*. We used a kinetic bioaccumulation model that incorporates geochemical conditions and physiological processes to predict bioaccumulation resulting from aqueous and dietary exposures. We first characterized the bioavailability of dissolved U(VI) in a series of controlled laboratory experiments at various water hardness, pH, and in the presence of dissolved natural organic matter (NOM) as a competing ligand. Results showed that dissolved U is bioavailable under all the geochemical conditions tested. In general, U bioavailability decreases with increasing pH, increasing Ca concentrations, and when NOM is present, suggesting the increase in concentration of strong aqueous U complexes limits U bioavailability. To characterize the bioavailability of dietborne U, we exposed *L. stagnalis* to benthic diatoms pre-exposed to a range of dissolved U concentrations, as well as to diatoms mixed with different forms of particulate U. We inferred U bioavailability from calculations of U assimilation into tissues. U assimilation efficiency (AE) ranged from 25 to 70%, indicating that U is bioavailable when ingested with food. Uranium was most bioavailable from diatoms with sorbed U and least bioavailable from leached contaminated soils collected near active breccia pipe U mine sites. Exposure to increasing concentrations of dietborne U for a given solid-phase did not impair the snail's digestive processes (AE did not vary among dietary concentrations), but did trigger a behavioral response (i.e., food avoidance), which is of significance to higher level processes like growth and reproduction. Model simulations using environmentally relevant exposures indicated that most of the bioaccumulated U is attained from dietborne exposure.

284 Experimentally derived acute and chronic copper Biotic Ligand Models for rainbow trout

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We evaluated the effects of varying water chemistry (pH, Ca, Mg and dissolved organic carbon concentrations) on the acute (96-h) and chronic (30-d) toxicity of waterborne Cu to juvenile rainbow trout (*Oncorhynchus mykiss*). Acute and chronic Biotic Ligand Models (BLMs) were developed from the experimentally-obtained toxicity data-sets. Our results indicate that Cu is predominantly an acute toxicant to rainbow trout, as there were no observable growth effects and the 96-h and 30-d LC50 values were similar. Calcium and DOM were greatly protective against both acute and chronic Cu toxicity, but Mg seemed to only protect against chronic toxicity. Additional protection occurred at pH 5 in acute exposure and at pH 8.5 in chronic exposure. In the range of water chemistry conditions tested, the observed 96-h LC50 and 30-d LC20 values varied by a factor of 39 and 27 respectively. The newly developed acute and chronic BLMs explained these variations reasonably well (i.e., within a 2-fold error), except at pH ≥ 8. The 96-h LC50 values of 53 out of 90 toxicity tests from 19 independent studies in the literature were reasonably well predicted by the new acute BLM. The LC20 predictions from the new chronic BLM were reasonable for 7 out of 14 toxicity tests from 6 independent chronic studies. A residual pH effect was also observed for both the acute and the chronic data-sets. The present study presents the first experimentally developed chronic Cu BLM for the rainbow trout. To the best of our knowledge, it also presents the first acute Cu BLM that is based on a published data-set for trout. [This work is funded with a CRD grant from NSERC, in partnership with ICA, CDA, ILA, IZA, NiPERA, Glencore, Teck Resources, and Vale].

285 Copper alters hypoxia sensitivity, performance and the behavioral emersion in the amphibious fish Kryptolebias marmoratus

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Elevated levels of metals have been reported in mangrove ecosystems worldwide, but there is little known regarding the impacts of these metals in the tropical mangrove environments on the behaviour and overall fitness of key mangrove species. One key behaviour employed by species such as *Kryptolebias marmoratus* (mangrove rivulus), is to leave water (emersion) in response to deteriorating conditions. In a laboratory experiment we showed fish exposed to environmental levels of waterborne copper (Cu) emersed at a higher dissolved oxygen level (7.5 ± 0.6%), relative to the control treatment group (5.8 ± 0.4%). In a follow-up study performed at Long Caye, Belize, waterborne Cu exposure also significantly altered jump performance and time to emersion when dissolved CO_2 was increased. Changes in emersion response were associated with changes in gill structure, with an increase in gill surface area and a reduced interlamellar cell mass (ILCM). Together these studies show that metals in mangrove settings have the capacity to interfere with key behaviours of the mangrove rivulus, effects that could impact survival.

286 A Mystery Tale: Nickel is Fickle when Snails Fail

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Recent studies (Niyogi et al. 2014, Aquat Tox. 150, 36-44; Nys et al. 2016, ETC 235, 1097-1106; Schlekat et al. 2010, Sci. Tot. Env. 408, 6148–6157) suggest that when assessed by the juvenile growth test, the freshwater snail *Lymnaea stagnalis* may be among the most sensitive of freshwater organisms to waterborne Ni. Indeed, Niyogi et al. (2014) reported an EC20 < 1.3 µg Ni L^{-1} in Miami tap water. In the course of a study on metal mixtures, we assessed the chronic toxicity of Ni alone to juvenile *L. stagnalis* in Vancouver tap water that had been amended to more closely match the chemical composition of Miami tap water. Chronic Ni toxicity in Vancouver water was almost two orders of magnitude lower (EC20 = 100-140 µg Ni L^{-1}) than reported in Miami water. In contrast, the toxicities of 3 other metals (Cu, Zn, and Pb) were very similar to values in the literature for Miami and other waters. To pursue this anomaly, we initiated a research program in which both snails and tap water were exchanged between Vancouver and Miami, with tests performed at the two sites focused on Ni uptake rates and chronic Ni toxicity. Additionally, fluorescence techniques were used to assess Ni binding by native dissolved organic matter (DOM) in the two waters. DOM fluorescence suggested the possibility that proteinaceous ligands occurred in Vancouver water, but not in Miami water. Results to date suggest a very complicated mystery, and that factors as yet unknown may attenuate the chronic toxicity of Ni to juvenile snails. This apparently high variability in sensitivity of *L. stagnalis* to Ni has significant implications for Ni risk assessments and water quality standard derivation (IZA, NiPERA, ICA, CDA, Rio Tinto, NSERC CRD).

287 What a sea cucumber and its energy metabolism can tell us about copper effects?

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Sea cucumbers are exclusively marine invertebrates found worldwide. Due to their association with the sea bottom, they can be exposed to metals through water or sediment and their gut can play an important role in metal contamination. Copper effect on freshwater organisms is a well known ionic and osmoregulatory disruption, but in marine osmoconformers the toxic effects of this metal are yet to be established. Some studies point to a respiratory or energy impairment in those animals, leading to questions about copper effects on the enzymes responsible for the energy production, although an effect on Na^{+}/K^{+}-ATPase (NKA) should not be ruled out. In the gut, NKA generates the gradient responsible for Na^{+} and glucose transport, and since it depends on ATP, could also be affected by impairment on energy production. This study aimed to evaluate the effects of copper on the energy metabolism [hexokinase (HK), phosphofructokinase (PFK), pyruvate kinase (PK), lactate dehydrogenase (LDH) and citrate synthase (CS) activities, ATP and glucose content], NKA activity and Na^{+} content of *Trachythyone crassipeda*, a sea cucumber found in Brazilian marine waters. Adult animals were exposed to 5, 9, and 20 mg Cu/L for 96h at 30 ppt. A control test (no copper added) was also run. Following exposure, sea cucumbers gut was dissected and collected for analyses. HK and CS activities were diminished in animals exposed to all concentrations of copper in relation to the non exposed. Exposure to 9 and 20 µg Cu/L caused a reduction in PK activity when compared to animals kept under control condition and those exposed to 5 µg Cu/L. LDH activity was reduced after exposure to 5 and 9 µg Cu/L in relation to non exposed organisms. Glucose content was elevated in animals exposed to all copper concentrations in relation to control animals. No differences were observed in PFK and NKA activities and ATP and Na^{+} contents between treatments. Since HK is less active, less glucose is phosphorylated, leading to a higher content of glucose in the exposed animals. Although CS activity is reduced implying in a smaller ATP production, ATP content remained unaltered, indicating that its cycling might be occurring with the aid of phosphagens. Our findings suggest that acute copper effects in osmoconforming animals can be related to energy metabolism impairment. Also, they indicate that sea cucumbers can be an interesting model to identify the mechanism underlying copper toxicity in marine invertebrates.

One Health: Opportunities for SETAC Leadership in Integrating Environmental, Human and Animal Health

288 One Health and Transdisciplinarity: Gaps in Science and Policy in Wildlife Health and Conservation

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According to WWF's Global Living Planet Report 2016, declines in vertebrate populations averaged 58% between 1970 and 2012. Humanity has much more to learn about the critical roles that these species play in both complex ecological processes and the health of humans. Wildlife is increasingly impacted by encroachment, malnutrition, toxicants, and emerging diseases shared with domestic animals and humans. Infectious and non-infectious diseases can be catastrophic to already stressed populations. Amphibian chytridiomycosis, white nose syndrome of bats, Tasmanian devil facial tumors, Ebola in great apes, nutrient-driven hypoxic dead zones impacting fish and shellfish, and mites, microbial pathogens and neonicotinoids in honeybees are a few examples of infectious and non-infectious agents becoming leading factors in species declines and extinctions. While compelling, the human health justification for wildlife conservation is extremely anthropocentric, and an urgent need is to address how biodiversity depends on us. One Health collaborations in research, intervention, and education should think on coincidental health problems and risks in areas of spatial and temporal overlap among species and stressors including—crowding, pathogens, metals, nutrients, biotoxins, anthropogenic chemicals, and climate change. Collectively countering such stressors can restore ecosystem services that protect humans and non-humans alike. Transdisciplinarity, integrative research, and capacity building are core elements in establishing One Health interventions that address extant, emerging, and re-emerging pathogens and toxicants that harm humans, wildlife, and other components of biodiversity. These should be both "bottom/local up" and "top/national/international down." Innovative participatory methodologies that operationalize knowledge flow among stakeholders should consensually and sustainably address major "real-life" problems that confront society. The One Health approach requires practical, sustainable and effective solutions with a keen understanding of local socio-economic factors as well as a solid grasp of complex regional, national and international health and environmental policies. One Health offers time-sensitive opportunities for practitioners to apply their expertise to give rise to simultaneous benefits for humans, animals, and the environment. Such nested efforts are underway and should be expanded and built upon with great determination and vigor.

289 Linking Indicators are a Key to Integrative Science

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SETAC's One Health program recognizes that as individual disciplines of science become more interconnected they become more powerful. This occurs not only as methods and insights from one discipline improve the capacity of another but also because disciplines whose predictive capacities interact potentially allow society to use science to address more difficult questions. For example, if we wish to know what the effect of a change in pesticide management means for people who eat fish, we need to meaningfully link a series of forecasts together. In this example: How do changes in policies lead to changes in exposures of fish to pesticides, which then results in changes in pesticide levels in fish? Next how does this result in changes of exposures of people and how does that lead to changes in human behaviors and ultimately to changes in human health? A key element of this set of linked questions is the information handed off from one forecast to the next. This presentation reports on a national interagency program to identify metrics and indicators for linking natural system outputs to social system outputs and to human wellbeing. Specification of these indicators is the key to asking how changes in ecosystems lead to changes in human well-being. Importantly our specification of the indicators is developed in a partnership between biophysical scientists and social scientists. In this partnership we have developed a process that enables us to specify these indicators. The foundation of the process is to recognize that people use, appreciate or enjoy ecosystems in diverse ways. We have identified a list of "beneficiaries". For a select subset of the members of this list we are identifying biophysical metrics and indicators for select ecosystems (streams, lakes, estuaries, wetlands, forests, agro ecosystems and coral reefs). In this presentation we'll illustrate this process for one beneficiary and one ecosystem. As our program provides information on these indicators that the questions science is able to address will become more useful. As social and natural scientists working together on a sustained collaborative basis they help will help to fulfill the transdisciplinary vision and provide more powerful science to benefit society.

290 Why a One-Health Approach to Mercury Knowledge Dissemination in Support of the Minamata Convention Is Critical

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Having now been ratified by more than 50 nations, the global Minamata Convention on Mercury is entering in force in 2017. This means the signatories must begin implementing the provisions of the Convention and after five years an evaluation of the effectiveness of the instrument will take place. Although metrics based on the provisions dealing with such matters as engineering controls on emisison sources, better management of storage and disposal sites, and phase-out of mercury ciontaining products are necessary, they are not sufficient. The UN Global Environment Facility - Scientific and Technical Advisory Panel, the UNEP Mercury Partnership - Fate and Transport Working Group, the Minamata Convention Secretariat, and SETAC have been working together to identify and implement a centralized platform for mercury knowledge sharing. Articles of the Convention that relate to human health and environmental quality are seen to be important to judge the Convention effectiveness. While the specifics of how these provisions will be dealt with are still in development, the concept of One Health and its implications for cohesive treatment of risk reduction to both huiman populations and environmental systems, including supporting fate and effects modeling frameworks, underscore the need for the knowledge platform to address information needs and provision in a consistent way. This presentation will provide a basis and rationale for why this holistic approach is appropriate and in fact essential, building on work completed to date, as well as specific activities being undertaken to implement the concept on the UN Environment Live Platform.

291 Of Whales and Men: Using a One Health Perspective to Understanding Chromium Pollution in Great Whales

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The concept of 'one' health posits that humans, wildlife and the general earth ecosystem share a common environment and, therefore, what affects the health of one group impacts the health of all groups. To put it more succinctly, our health is intrinsically connected to the health of the species around us and so a more complete cross-species study of a toxicant will reveal more about its health impacts and how to address or prevent them. However, while toxicological impacts on human health are readily investigated, toxicological-impact studies in wildlife and domesticated species lag far behind. We have been pioneering a 'One Health' approach in carcinogenesis using chromium as a model toxicant. Hexavalent chromium [Cr(VI)] is a well-established genotoxicant and carcinogen in humans and laboratory animals. By contrast, its impacts in wildlife are rarely studied. Using great whale species such as the Northern right whale (*Eubalaena glacialis*), the Southern right whale (*Eubalaena australis*), the bowhead whale (*Balaena mysticetus*) and the sperm whale (*Physeter macrocephalus*), we discovered that whale Cr skin levels are dramatically elevated in whales from even the most remote ocean locations. In some individuals, Cr levels were remarkably high and are comparable to levels only seen in lung tissue from workers with Cr-induced lung cancer. Whales are exposed to Cr(VI) through both water and air, and thus, likely encounter both particulate and soluble forms of Cr(VI). In humans, the particulate form is more potent, though the effects of particulate Cr(VI) appear to be mediated through the release of soluble chromate ions. We investigated the cytotoxic and genotoxic effects of both soluble and particulate Cr(VI) in whale skin fibroblasts. Cytotoxicity was measured by clonogenic survival assay and genotoxicity was measured as production of chromosome aberrations. Both forms of Cr(VI) induced concentration-dependent increases in cytotoxicity and genotoxicity indicating that Cr(VI) is toxic to whale cells. Comparing the effects in whale cells to those in human cells revealed whales may be resistant to the genotoxic effects of Cr. Thus, consistent with improved health and understanding under a 'One Environmental Health approach' – wildlife data suggest that Cr has become a global pollutant spread by wind, water and biological currents, while human data suggest whales may have evolved a mechanism to protect against Cr(VI)-induced genotoxicity and carcinogenesis.

292 Evaluation of Anthropogenic Micropollutants in Waters and Subsistence Species Used by the Minnesota Chippewa

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Although the One Health Paradigm - a holistic, collaborative, systems-based approach to health research and policy - currently enjoys a wave of energy and funding, most of the emphasis to date has been on zoonotic and emerging infectious diseases. Models that emphasize chronic diseases and ecotoxicology are needed. Through a unique collaboration between the Minnesota Chippewa Tribe, Grand Portage Indian Reservation, University of Minnesota's College of Veterinary Medicine's Ecosystem Health Division and School of Public Health, Minnesota Pollution Control Agency, and the US Environmental Protection Agency, we are assessing threats by chemical pollutants to the sustainable use of tribal natural resources within key lacustrine habitats and fish species used by the Minnesota Chippewa, possibly threatening the very culture of the Minnesota tribes using these resources. This project is unique in that it is focused on system-wide exposures and responses across a variety of aquatic systems and subsistence fish species in the Lake Superior watershed, an understudied location. Initial efforts have focused on monitoring the presence of over 150 heavy metals, endocrine active chemicals, pharmaceuticals, and other commercially available chemicals within the tissues of subsistence fish species, waters, and sediment. In 2016, at least one chemical was detected in all water, sediment, and fish samples from fourteen locations, with a range of 3-10 chemicals found in fish, 1-30 chemicals found in sediment, and 3-64 chemicals found in water. Chemicals were detected at ng/l levels. one site, 64 chemicals were detected in water, 30 in sediment, and 7 in fish. Some chemicals detected in fish were detected across a large percentage of sites; betamethasone, fluticasone propionate, amitriptyline, and DEET were detected in fish from more than 60% of the sites. The results of this work will assist in guiding land and chemical management practices and promote a sustainable food and ecosystem within the Grand Portage Indian Reservation.

293 Healthy fish, healthy people: Using fish to study ecological and health impacts of early life exposures to chemicals on metabolic & bone development

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Fish inhabiting the New Bedford Harbor (NBH), Massachusetts, marine Superfund site can serve both as biological models for contaminant effects and indicators of human dietary exposure, contributing importantly to the assessment of ecological and human health risks of contaminant exposure. Polychlorinated biphenyls (PCB) and tributyltin (TBT) are bioaccumulative contaminants associated with NBH industries that belong to a growing class of metabolism-disrupting compounds believed to contribute to obesity, liver steatosis, and Type 2 diabetes in humans and other species. Here we show that embryonic exposure to PCBs and TBT produced phenotypic abnormalities and altered the expression of genes related to metabolic homeostasis in laboratory-reared killifish (*Fundulus heteroclitus*), an ecologically-important NBH fish. These biological effects suggest perturbations to metabolic and bone homeostasis in fish, consistent with effects seen in mammalian species; future transcriptomic analyses will provide insight into the underlying molecular mechanisms of toxicity for these compounds in fish. In a complementary investigation, we also used information from NBH seafood as a proxy for human dietary exposure to harbor-based Superfund chemicals, and show here that PCBs in human-consumed species from NBH have generally declined since 2003. This information may be useful in understanding the contribution of chemical metabolic disruptors in human obesity and metabolic disease. The combination of mechanistic studies using fish and the assessment of potential human exposure through consumption of contaminated seafood provides an effective and holistic approach to characterize both ecological and human health risks of exposure to environmental chemicals, including those frequently found at sites highly contaminated with multiple Superfund chemicals.

294 Using prior knowledge and local issues to develop a framework for assessment of sentinel species

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Environmental sentinels provide valuable information regarding organism-environment interactions as well as information related to human health. Many sentinel species are well-known indicators for environmental and human health (i.e. marine mammals). However, new and emerging environmental issues may not always be relevant to the known sentinel in each ecosystem. Using previous work to guide our assessment of a local sentinel, we can develop a framework that can be used for assessment of sentinel species for use with a new/different class of contaminants. The framework details a non-targeted analysis for the class of contaminants of interest, their bio-distribution within the sentinel, seasonal variability according to sampling regimes, determination of biological time points of interest for the contaminant, as well as feasibility of using the time points for monitoring and exposure assessment. This framework can be applied to a known sentinel for endocrine disrupting compounds (EDCs) in the southeastern United States, the American alligator (*Alligator mississippiensis*), for its utility as a sentinel of trace element exposure, specifically mercury. Mercury has been a problematic contaminant in the southeastern United States due to local point sources and atmospheric deposition, however, no sentinel species has emerged as a model organism for this exposure. The American alligator would make an ideal sentinel, as routine sampling is conducted for EDC exposure. Using the presented framework, this attractive opportunity is assessed for its utility in monitoring mercury contamination in the environment.

295 Integrating Human Health and Ecological Integrity into a Systems Framework

W. Fisher, USEPA/ Office of Research and Development; S.H. Yee, USEPA / Gulf Ecology Division

Unintended and long-term effects of environmental decisions on public health, social welfare and economic stability are difficult to understand, much less anticipate. This is partly because environmental decisions are too often considered separate from factors that determine individual and community well-being. To better understand the ramifications of a decision, a systems context is essential, where environmental, social and economic factors are considered simultaneously. Yet, the complexity of multiple interacting factors in a system is daunting, and has constrained effective systems thinking. Conceptual frameworks are useful for capturing, visualizing and organizing the connections among key factors in a complex system, and can be useful in advancing the OneHealth context. To this end, we merged the Driving force–Pressure–State– Impact–Response (DPSIR) structure for environmental resources and the Driving forces-Pressure-State-Exposure-Effect-Action (DPSEEA) structure for human health assessments to integrate the multiple social-cultural, economic and environmental aspects of decisions into an integrated EcoHealth DPSIR framework. The parallels within the DPSIR and DPSEEA structures provide a conceptual simplicity to the EcoHealth DPSIR. *Driving force* includes both economic sectors and social drivers; *Pressure* includes environmental stresses and human behavior; *State* includes environmental and human condition; *Impact* includes ecosystem services and human well-being; and Responses can include environmental or behavioral actions. The framework provides a visual organizational tool can be used at every step of a decision process, and can be particularly effective for complex, multidisciplinary issues that require a robust understanding of the linkages across ecological, health, social and economic disciplines.

Fate of Chlorinated Persistent Organic Contaminants in the Urban Water Cycle

296 Presence of polychlorinated biphenyls in the urban water cycle - legacy contaminants or current sources?

B. Kjellerup, R. Jin, S. Cao, S.C. Fusi, University of Maryland at College Park / Civil and Environmental Engineering

Stormwater runoff has been implicated as a major cause of recontamination of sediment near stormwater discharge points in urban water sheds as well as Department of Defense sites. Removal of persistent organic pollutants (POPs), specifically polychlorinated biphenyls (PCBs) from stormwater, thus preventing or significantly reducing the discharge to urban discharge areas including aquatic sediments is a priority due to the ability of these contaminants to enter the food chain, where they can present potent toxic and carcinogenic properties. Therefore, *new and innovative treatment media or mixes of media* to provide optimum removal of POPs with focus on PCBs and PAHs must be developed for stormwater runoff. In a current project emphasis has been placed on removal of dissolved contaminants. Subsequent removal of adsorbed POPs by microbial degradation through a "treatment train" stormwater best management practice (BMP) was also evaluated. The potential effect of stormwater containing PCBs on the sediment quality was evaluated for multiple locations in Baltimore Harbor, where sediment core samples were evaluated and compared to historical PCB concentrations. Also, current strategies for bioremediation of PCBs in stormwater retention cells and well as in sediment were evaluated.

297 Why are PCBs and PCDD/Fs dechlorinated by bacteria in some places but not others?

L.A. Rodenburg, Rutgers University / Environmental Sciences

In 2010, our research group published a paper in which we presented evidence that PCBs are dechlorinated by bacteria in sewers, landfills, and contaminated groundwater in the Delaware River basin. Since then, we have found evidence that PCDD/Fs are dechlorinated along with PCBs in all three of these urban infrastructure categories in locations from the west coast of the US (Portland Harbor Superfund Site) to the east coast (New York/New Jersey Harbor) in freshwater ecosystems as well as some estuarine systems. But we have also found places where dechlorination does not occur, even though PCB and/or PCDD/F concentrations are high, such as stormwater in Santa Fe, NM; sewers in Spokane County, WA; and groundwater in the Lower Duwamish Waterway superfund site. We will present the evidence for dechlorination (or lack thereof) in these system and examine some of the factors (salinity, groundwater and wastewater residence time) that may explain why dechlorination happens in some places but not others. We will also examine the dechlorination pathways and how they may differ from one location to the next.

298 Microbial Impacts from the Presence of Triclosan and Triclocarban in Sediments and Wastewater Sludges

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Triclosan and triclocarban are pervasive chlorinated contaminants in wastewater treatment systems and immediate downstream soils and sediments. Three different studies have elucidated how these compounds significantly alter microbial communities: 1. A two-year long batch experiment with sediment testing triclosan biodegradability and the associated microbial community impacts under a high dosage of triclosan, 2. A one-year long draw-and-fill experiment with anaerobic digester sludge with typical triclocarban and triclosan concentrations testing biodegradability and microbial impacts under physical/chemical variables, and 3. A three-month-long anaerobic digester reactor experiment independently testing the effects of both compounds under low, medium, and high dosage regiments on microbial community composition. In all cases, large numbers of bacterial species were suppressed, often including putative organohalide respiring groups, while other bacteria commonly associated with antibacterial resistance were significantly enriched. Antibiotic resistance genes were found to increase in abundance, and triclosan and triclocarban, when measured, were not sufficiently degraded. This work indicates that triclosan and triclocarban affects on microbial communities are long-lasting and concentration-dependent.

299 Biofilm covered activated carbon particles: Application as a microbial inoculum delivery system

S. Capozzi, University of Maryland at College Park / Environmental Science; B. Kjellerup, A. Prieto, S. Saffari Ghandehari, C. Bodenreider, University of Maryland College Park / Civil and Environmental Engineering

Previous research demonstrated enhanced dechlorination of Aroclor 1248 in mesocosm studies when biofilm covered activated carbon particles were applied as a delivery system for bioaugmentation. Here, we further investigate the fundamental characteristics of the materials and the interaction with biofilm that enhance the dechlorination process. Preliminary mesocosm experiments are assembled using commercially available activated carbon materials and an anaerobic microbial consortium including dechlorinating bacteria. Experiments are performed in mesocosms containing various activated carbon materials using 90 mL of minimal medium with lactate as the electron donor and tetrachloroethylene as the electron acceptor. Tetrachloroethylene and dechlorination products (i.e., trichloroethylene, dichloroethylene, vinyl chloride and ethene) are detected using gas chromatography coupled to a flame ionization detector (GC/FID). The number of bacteria are measured using quantitative polymerase chain reaction (qPCR) and biofilm characteristics are monitored with confocal microscopy and scanning electron microscopy (SEM). Future research is aimed at identifying the mechanism responsible for the observed enhanced dechlorination, which may include sorption, electrical conductivity, surface area and porosity.

300 Bioretention capture efficiency of PCBs from stormwater

R.A. Jack, King County / Natural Resources and Parks

Stormwater has been identified as a significant source of PCBs to numerous Washington State waterbodies including Lake Washington, the Duwamish River, and the Spokane River. These loads have led to 14 fish advisories; many recommending no consumption at all. Western Washington Municipal Stormwater Permits require the use of Low Impact Development (LID) where feasible and bioretention is a commonly utilized LID best management practice (BMP) in Western Washington. For Washington State stormwater design purposes, the default bioretention soil mixture (BSM) is 60% sand, 40% compost (Ecology, 2012). This study is evaluating the degree which default BSM removes polychlorinated biphenyls (PCBs) from stormwater, and estimating the efficacy of capture and retention of PCBs over a two-year period. The study uses

208L barrel mesocosms with both BSM alone and BSM plus plant treatments each replicated three times. The ultimate objective is to develop a better understanding of how well BSM captures and retains PCBs across seasons over a two-year period. The project is addressing the following questions: What is the PCB removal (capture) rate for BSM by storm, and does it vary by congener? What is the wet season PCB sequestration (retention over multiple storm events) in BSM, and does this vary by congener? Compare sequestered mass of PCBs with estimated stormwater loads. What is the PCB retention in BSM during the dry season, and does it vary by congener? Because the chemical properties of PCBs vary by congener, evaluation of PCB losses from the mesocosms through volatilization or outflows are being developed on both an individual congener basis, as well as total PCBs (sum of detected congeners). The congener specific mass balance provides a conceptual model of PCB congener behavior in mesocom bioretention cells with and without plants. Combined, the conceptual model and mass balance will describe the potential effectiveness of Western Washington bioretention cells to reduce PCB loadings to receiving water bodies. PCB degradation in soils is minimal, but degradation rates in the conceptual model may be estimated using published literature. Ensuring that bioretention BMPs address PCBs, a stormwater pollutant of high public health concern, is important to ensure that investments in retrofits reduce the circulation of PCBs in the environment.

301 Biochar-amended Biofilters for Removal of Persistent Organic Contaminants from Urban Stormwater

B. Ulrich, Eawag - Swiss federal Institute of Aquatic Science and Technology / Environmental Chemistry; C.P. Higgins, Colorado School of Mines / Civil Environmental Engineering

Urban stormwater runoff has led to the contamination of receiving waters with a variety of persistent chlorinated organic contaminants, including urban-use pesticides (e.g., fipronil), herbicides (e.g., diuron), and flame retardants (e.g., tris(1,3-dichloro-2-propyl)phosphate, TCPP). While Low Impact Development (LID) systems have been shown to effectively remove many conventional contaminants from runoff (e.g., petroleum hydrocarbons), they have proven less effective for removal of these more polar, chlorinated organic contaminants. The objective of this study was to develop and assess a novel, cost-effective technology for removal of persistent organic contaminants from urban stormwater during infiltration. It was hypothesized that amendment of infiltration systems with biochar would enhance the sorption of contaminants, and that additional amendment with organic carbon sources (i.e., compost) would enhance biodegradation and prevent accumulation. Efforts were undertaken to assess the potential performance improvements for such biochar-amended stormwater biofilters relative to conventional systems; beginning from the conceptual phase by evaluating the processes driving contaminant removal (i.e., sorption and biodegradation), and concluding with an assessment of contaminant removal performance under realistic operating conditions. The findings of this study demonstrated remarkably improved removal of organic contaminants in stormwater biofilters amended with biochar, and also indicated potentially improved removal of other more conventional contaminants (e.g., nutrients); motivating the application of full-scale biochar-amended biofilters to achieve improved urban water quality. Moreover, this potentially cost-effective technology may promote sustainable stormwater management practices by enabling practitioners to pro-actively treat runoff for organic contaminants while improving contaminant removal more broadly.

302 Dechlorination Processes in Urban Salt-Impacted Lakes

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Many northern urban lakes have increased chloride concentrations as a result of seasonal road salt input. This chloride can be incorporated into the natural organic matter present to form chlorinated natural organic matter (Cl-NOM). Literature evidence has shown that bacteria are capable of degrading Cl-NOM in some of the same ways that they degrade chlorinated persistent organic contaminants. A better understanding of dechlorinating bacteria and the dechlorination processes active in uncontaminated lakes should provide insight into improving remediation efforts, especially at low contaminant concentrations where processes in uncontaminated environments have evolved to perform well but where degradation may stall in contaminated environments. In this work, sediment sampling/analysis and lab enrichment studies were used to determine how different concentrations of Cl-NOM effect the dechlorinating microbial populations and functional genes present in urban lakes. The metagenomes of five lake sediments with different chloride and NOM concentrations were sequenced to analyze the dehalogenase genes present. Sediment from a rural lake and a salt impacted lake were also enriched with different concentrations of Cl-NOM and carbon/electron donors to monitor the microbial populations and compare the dechlorination mechanisms under different conditions and within different lake sediments. Preliminary results showed that dehalogenase genes (as opposed to reductive dehalogenase genes) were the dominant dechlorination enzymes present in all of the lake sediments sampled and were present at a higher frequency in the salt impacted lakes. These genes, while active in dechlorination, do not serve as a mechanism by which bacteria can gain energy for growth. The enrichment study of lake sediments with high concentrations of carbon and Cl-NOM showed an increase in the reductive dehalogenase genes present, although this work is ongoing. It is expected that dehalogenase genes will be more dominant under nutrient limited conditions because these conditions may not be able to support the growth of bacteria capable of energy generation via only reductive dechlorination. To date, remediation of chlorinated contaminants has focused on reductive dechlorination; nevertheless, these results show that the role of other dehalogenase genes in the dechlorination of Cl-NOM and chlorinated persistent organic contaminants should not be overlooked, particularly in urban salt impacted lakes.

303 Biologically mediated sulfidation of Fe(0) materials for enhanced dechlorination of trichloroethene

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Surface sulfidated zero-valent iron [Fe(0)] particles represent a promising engineered approach for the degradation of persistent halogenated contaminants such as chlorinated ethenes. The dechlorination activity of these particles is attributed to their reactive sulfide surfaces, which enhances redox activity and increases resistance to deactivation. Sulfidation of Fe(0) is commonly achieved via aqueous chemical treatment methods. The potential of increasing Fe(0) dechlorination activity via biologically produced sulfur species has not been assessed. The current study explored whether reactive sulfided iron surfaces for trichloroethene (TCE) reduction could be generated using sulfate-reducing bacteria (SRB), which are present in many waters and can be readily enriched. Batch experiments utilizing *Desulfovibrio desulfuricans* as a model sulfate reducer were conducted in two stages. Untreated Fe(0) particles were first exposed to buffered solutions of differing chemical compositions, with and without *D. desulfuricans*. After various exposure periods, TCE was amended and dechlorination activity assessed. Preliminary results showed that biologically generated sulfided surfaces formed on iron particles, and these particles demonstrated enhanced activity compared with untreated controls. Rates of dechlorination activity were dependent upon concentrations of biologically produced sulfide and common background electrolytes. This strategy of using SRB to generate environmentally compatible reactive surfaces for dechlorination is potentially advantageous over traditional biological approaches, as sulfate reducers are generally more robust and grow more readily than organohalide respiring bacteria.

Fate, Toxicology or Risk Assessment of Materials of Interest to the Military

304 Potential role of nitrocellulose in the release of energetic materials from fired munition residues

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Munition residues dispersed on military ranges and training grounds cause environmental and public health concerns as they can be leached by precipitation or surface runoffs. Binding to soil matrix has been the primary focus of the subsurface fate and mobility of residue-bound energetic materials (EMs). This study examines the potential role of nitrocellulose – a common binding matrix in propellants – in the release of residue-bound EMs. Month-long batch experiments were performed to quantify the sorption capacities of military grade nitrocellulose for nitroglycerin (NG) and 2,4-dinitrotoluene (2,4-DNT), two common EMs. The binding of NG and 2,4-DNT were nonlinear, with isotherms determined as $S = 250C^{0.916}$ and $S = 1200C^{0.668}$, respectively (S and C in mg/kg and mg/L). When normalized against organic carbon content, nitrocellulose was 10 times stronger than soil organic carbon in binding both NG and 2,4-DNT. This suggests that nitrocellulose may slow down the overall release of propellant-EMs through retarded diffusion within the residues. The nonlinearity of the isotherm also implies that stronger retardation can be expected at the latter phases of leaching. The observed affinity of nitrocellulose for NG and 2,4-DNT may explain the declining dissolution of propellant-EMs over time and the very slow leaching of EMs from unspent residues through soil columns. Adsorption and desorption kinetic data for both NG and 2,4-DNT were adequately interpreted with an intraparticle-diffusion model with effective diffusivity as the sole fitting parameter. The kinetic modeling results further reinforce the potential role of nitrocellulose in influencing EM leachability. Implications of nitrocellulose binding on risk assessment and remediation of EM-impacted areas will be elaborated.

305 Predicting the Rate of Abiotic Reduction of Munitions Constituents Using the Reaction Energy of A Hydrogen Atom Addition

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The abiotic reduction rate of munitions constituents (MC) in general and nitroaromatic compounds (NAC) in particular varies dramatically over multiple orders of magnitudes with respect to the MC, functional group variations within a class of compound, and the properties of the reductant. In the development of a quantitative structure activity relationship (QSAR) to predict the abiotic reduction rate constant, the usual parameter that is used for the NAC is the one electron reduction potential. This measures the energy required to transfer an electron from the reductant to the NAC: $NAC + e^- \rightarrow NAC^-$. We will present a new QSAR that uses the energy of transferring both an electron and a proton, i.e. the energy of transferring a hydrogen atom from the reductant to the NAC. The reaction is $NAC + H \rightarrow H\text{-}NAC$, where H-NAC is the radical formed by the addition of the H atom to the nitro group. The energy of this reaction is estimated using high level quantum chemical computations. The prediction errors for the QSARs using both the electron and hydrogen addition reactions will be compared, for a number of NACs and reductants. We have found that the prediction error is substantially reduced when the hydrogen atom reaction energy is used. This methodology can be used to estimate the relative reactivity of many nitroaromatic and other nitrogen-containing MCs based on molecular structure only, since the required parameter: the energy of a hydrogen atom addition, is computed using quantum chemistry. We expect that this methodology will be particularly useful in evaluating the degradability via abiotic reduction of classes of MC in the early stages of development.

306 Evaluating the Relationship between Soil Properties and Environmental Fate and Transport of Insensitive Munitions

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Insensitive munitions (IM) are of interest to the Army due to the increased safety and detonation control that is provided by the IM compared to traditional munitions. The environmental fate and transport data of these compounds is of interest to gain an understanding of the environmental impact and the potential risks associated with using specific materials of interest. Current modeling efforts include environmental fate and transport data, however the accuracy for predicting the fate and transport within soils can be improved upon. Soils are inherently heterogeneous both in terms of chemical composition and function which control the processes such as degradation rates and the ability of transport within a system. The differences between 5 soil taxonomic orders and the soil physical/chemical properties within the orders that impact environmental fate and transport were evaluated. The sorption coefficient of both individual constituents and an insensitive munition formulation were determined using soils from all 5 orders. Sorption coefficients were evaluated due to their twofold benefits for providing a basic understanding of the potential transport of a material and incorporation into environmental modeling for predicting environmental impacts. Results across all 5 soil taxonomic orders suggest that the formulation does not have a linear relationship with the individual components. In addition, properties related to soil fertility impact sorption more than properties related to the taxonomic order. Results from this research can provide a better understanding of which soil physical/chemical properties are most important in relation to taxonomic order allowing for improvement of predictive models for both easily accessible areas that can be sampled/measured as well as areas where prediction is the only available option.

307 Mineral-Mediated Attenuation of Nitroaromatic Contaminants in Groundwater Systems

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Contamination in soil, sediment, and groundwater by nitroaromatic compounds is of significant concern at military munitions production, storage, and disposal sites. In the subsurface, attenuation of these contaminants is typically mediated by iron-bearing minerals in the form of oxides and sulfides. Here, we present the results of abiotic mineral-mediated attenuation of the insensitive munition, 2,4-dinitroanisole (DNAN), and the munition precursor, 2,4-dinitrotoluene (DNT). Degradation rates are provided for DNAN and DNT in suspensions of goethite (α-FeOOH), magnetite (Fe_3O_4), and mackinawite (FeS). Analysis by compound specific isotope analysis (CSIA) was performed to characterize the isotopic signature and kinetic isotope effect of the fractionation processes. Our results demonstrate the efficacy of mineral-mediated attenuation and its level of sensitivity to a range of environmental factors.

308 Toxicity of insensitive munitions mixture formulations to the amphipod Hyalella azteca in sub-chronic and chronic water-only exposures

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Within the US military, new insensitive munitions (IMs) are rapidly replacing conventional munitions improving safety from unintended detonation. Toxicity data for IM chemicals are expanding rapidly, however IM constituents are typically deployed in mixture formulations, and very little is known about their mixture toxicology. In the present study we sought to characterize the mixture effects and toxicology of the IM formulations IMX-101 (mixture of 2,4-dinitroanisole [DNAN], 3-nitro-1,2,4-triazol-5-one [NTO], and nitroguanidine [NQ]) and IMX-104 (DNAN, NTO, and hexahydro-1,3,5-trinitro-1,3,5-triazine [RDX]) in sub-chronic (10 d) and chronic (35 d) water-only *Hyalella azteca* toxicity tests. Endpoints were survival, growth and reproduction (as young/female; 35-d test only). In 10-d exposures, DNAN was the most potent constituent, eliciting an LC50 of 16.0 mg/L; the LC50 for NTO and NQ were 891 and 565 mg/L, respectively and RDX did not elicit significant mortality up to 32.8 mg/L, near its solubility. The toxicity of IMX-101 was driven by the effective concentration of DNAN, with NTO and NQ not appreciably influencing the toxicity of DNAN. However, the presence of NTO, RDX or both elicited interactive effects causing significantly decreased toxicity of DNAN in 10-d exposure to IMX-104. Significant sublethal growth reduction was observed in 10-d exposures for DNAN, IMX-101 and IMX-104, but not for NQ, NTO, or RDX. Longer exposure (35 d) to IMX-101, IMX-104, DNAN and RDX resulted in higher responsiveness of the survival endpoint compared to 10-d exposures. However, the responsiveness of sublethal endpoints was greater than that for survival for DNAN but not for RDX, IMX-101 and IMX-104 in 35-d exposures.

309 Transcriptomics Provides Mechanistic Indicators of Mixture Toxicology for IMX-101 and IMX-104 Formulations in Fathead Minnows

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The mixture effects and toxicology of the two predominant insensitive munitions (IM) formulations IMX-101 and IMX-104 were characterized in acute (96h) larval fathead minnow (*Pimephales promelas*) exposures. IMX-101 consists of 2,4-dinitroanisole (DNAN), 3-nitro-1,2,4-triazol-5-one (NTO), and nitroguanidine (NQ) while IMX-104 is composed of DNAN, NTO, and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). DNAN was the most potent constituent in IMX-101 eliciting an LC50 of 36.1 mg/L, whereas NTO and NQ did not elicit significant mortality in exposures up to 1,040 and 2,640 mg/L, respectively. IMX-101 elicited toxicity representative of the component concentration of DNAN within the mixture where toxic-unit (TU) calculations indicated non-interactive toxicity among constituents. Transcriptomic responses for individual IMs indicated unique functional responses characteristic of: oxidative stress, impaired energy metabolism, tissue damage, and inflammatory responses in DNAN exposures; impaired steroid biosynthesis and developmental cell-signaling in NQ exposures; and altered mitogen-activated protein kinase signaling in NTO exposures. Transcriptional responses to the IMX-101 mixture were driven by the fractional equivalent of DNAN. Given that the functional responses among the individual constituents of IMX-101 were unique, and NTO and NQ did not interact with DNAN within the IMX-101 mixture exposure, the overall toxicity and toxicogenomic responses indicated that the mixture toxicity of affective exposures to the IMX-101 formulation were “independent”. Alternatively, the toxic response to the IMX-104 exposure indicated at least slight potential for toxicological synergism where the toxicity was 18% greater than expected compared to the ΣTU for DNAN, RDX and NTO. Functional transcriptomic responses to DNAN were conserved in the IMX-104 exposures, however with less fidelity compared to IMX-101. Based on previous transcriptomics responses to acute RDX exposures in fathead minnow larvae, we hypothesize that the potentially synergistic responses within the IMX-104 mixture are related to interactive effects of each DNAN and RDX on systemic energy metabolism.

310 Safe Environmental Exposure Levels For RDX and TNT Using Toxicogenomic Data

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The US Army benefits from improved toxicity testing procedures, using relevant models that are cheaper, and higher throughput than current approaches. In addition, through the development of Causal Adverse Outcome Pathway Networks (CAOPNs), we are beginning to identify a smaller number of assays and biomarkers that are predictive of toxicity, without requiring testing of every key event within an Adverse Outcome Pathway (AOP). Here we will describe our work using Daphnia, C. elegans, and zebrafish embryos to estimate points of departure (PODs) for RDX and TNT based on relevant toxicity endpoints (i.e., carapace remodeling for daphnia, cuticle molting for C. elegans, and neurotoxicity and cuticle remodeling for zebrafish). In addition, we used human induced pluripotent stem cell hepatocytes (iPSC-hepatocytes) to estimate a POD for TNT based on oncotic necrosis. We then coverted the PODs to reference doses (RfD; mg/kg-day). For the iPSC-hepatocytes we used a one-compartment reverse toxicokinetics model with computationally estimated fraction unbound estimates to estimate the RfD. If we use our aquatic species as surrogates for human RDX exposure and toxicity, then we estimate an RfD of 0.011 mg/kg-day for RDX. For TNT, we estimate an RfD of 0.335 mg/kg-day based on the iPSC-hepatocytes and 10x uncertainty for human variability. By using this in vitro model we were able to take off 100x uncertainty from the USEPA and ATSDR RfDs. This places our RfD within an order of magnitude of the original POD for EPA and ATSDR, which was estimated in dogs. This demonstrates that toxicogenomic data when coupled with CAOPNs may be suitable for estimating safe environmental exposure levels.

311 Ecotoxicology of Aggressively UV-degraded Insensitive Munitions

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The improved safety from unintended detonation of insensitive munitions (IMs) is a benefit to the Army Forces. Insensitive munition IMX-101 has moved to production as a mixture of three constituents; nitrotriazolone (NTO), nitroguanidine (NQ) and dinitroanisole (DNAN). We demonstrated enhanced phototoxicity of IMX-101 to *Ceriodaphnia dubia* but did not identify the cause. Due to the complications in photochemistry and mixtures toxicology, we conducted additional testing using *Daphnia pulex* due to its better defined genome to allow forensic toxicogenomics. Our current research with *D. pulex* provided additional evidence that the baseline toxicity of DNAN (48-h LC50 = 25 mg/L) is greater than neutralized NTO (LC50 = 1235 mg/L) and NQ (LC50 = 1486 mg/L). The IMX-101 mixture toxicity (LC50 = 63 mg/L) was intermediate between its constituents. These data indicate *D. pulex* was more sensitive to IMs than *C. dubia*. Generally, these undegraded IMs are considered moderately toxic to practically nontoxic, and data suggest they are less toxic than the trinitrotoluene (TNT) they are to replace. A worst case scenario was created by placing dissolved IMs into a photo reactor for the equivalent of 24-h UV and visible light exposure. Notable increases in toxicity were observed for NQ (100 to 1000X), NTO (10 to 25X) and IMX-101 (20X). Photo-enhanced toxicity was not observed for DNAN. It is critically important to understand that both theinitial concentration and photodegradation conditions have strong influence on the relative increase in toxicity.NQ appears to drive the toxicity of the photo-degraded IMX-101 mixture. Experiments on the photo-kineticdegradation rates of NQ suggested rapid degradation, with much of its maximal toxicity observed after less than 60minutes. Wet chemistry and computational methods are being employed to determine the role of differentdegradation compounds, such as but not limited to nitrosoguanidine, nitrate, nitrite, urea, ammonia, cyanamideand cyanoguanidine. Tissues were submitted for transcriptomic assays and the integration of ecotoxicology,photo-chemistry and genomic results are being employed to identify the source of UV-enhanced toxicity andthe mechanism by which the enhanced toxicity occurs. Further work pursues environmentally realistic photodeg-radationand hazard experiments in mesocosms.

Aquatic Toxicology and Ecology – Part 2

312 Acute toxicity of three anticoagulant rodenticides to various fish species

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Rodent eradication projects have utilized bait pellets containing antico-agulant rodenticides (ARs). Direct and secondary poisoning of non-target organisms such as birds, mammals, and reptiles, have been an associated risk of AR use. The evaluation of risk to non-target species has been an integral part of the rat eradication and control programs by U.S. Fish and Wildlife Service. Due to public and regulatory concerns regarding anti-coagulants, risk to non-target fish species associated with bait broadcast areas needed to be addressed. Therefore, our objectives were to determine median lethal dose (LD50) range estimates (µg chemical per gram of body weight) of three AR chemicals (diphacinone, chlorophacinone, and brodifacoum) in freshwater and saltwater species based on intraperito-neal injections. The LD50 values for ARs in freshwater fish ranged as follows: diphacinone (165-303 µg/g), chlorophacinone (180-402 µg/g) and brodifacoum (46-100 µg/g). The AR LD50 ranges in saltwater fish were: diphacinone (90-175 µg/g), chlorophacinone (125-182 µ/g) and brodifa-coum (36-75 µg/g). Tissue concentrations of ARs in the liver were used to confirm exposure and assess depuration and metabolism of each AR in the test species. Estimated half-lives in the liver show a rapid decrease in AR concentration. In comparison to other non-target and target species, fish species are less sensitive to anticoagulant rodenticides. The results from our study indicate that the risk of direct toxicity from ARs on non-target fish species during rat eradication efforts should be minimal.

313 The influence of water chemistry and gill physiology on the uptake and sensitivity to TFM, for juvenile lake sturgeon

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Presentation Type: Platform Preferred Track: Aquatic Toxicology and Ecology Session: Aquatic Toxicology and Ecology Abstract Title: The influence of water chemistry and gill physiology on the uptake and sensitivity to TFM, for juvenile lake sturgeon Authors: [1]S. Hepditch, [1]O. Birceanu, [2]L. O'Connor, [1]J.M. Wilson, [1]M.P. Wilkie. [1]Wilfrid Laurier University and [2]Department of Fisheries and Oceans Canada ABSTRACT: Application of the lampricide 3-trifluomethyl-4-nitrophenol (TFM) to control the invasive sea lamprey (Petromyzon marinus) within the Laurentian Great Lakes seldom causes non-target mortality. However, under certain conditions, TFM can harm species such as the lake sturgeon (Acipenser fulvivens). Juvenile lake sturgeon are vulnerable to TFM-induced mortality, particularly when smaller than 10cm in length, and their sensitivity increases in waters of higher alkalinity, which contrasts with the reductions in toxicity observed for sea lamprey and teleost fishes. The rationale behind the sensitivity to TFM for the juvenile sturgeon has not been elucidated. However, lake sturgeon population levels remain depressed at 1-2% of their historic levels, making it imperative to better understand why juvenile lake sturgeon are more sensitive to TFM, and how their susceptibility is influenced by water chemistry. To determine if water chemistry affects rates of TFM uptake by sturgeon, the rates TFM uptake at varying pH and alkalinities by smaller (< 10 cm) and larger (>150 mm) lake sturgeon were measured using 14C-TFM. We hypothesize that water chemistry influences the morphology of the gill, therefore changing the rate at which the lampricide is taken up by the fish and its effects on the physiology of the animal. Contrary to field studies, which have reported increases in TFM sensitivity with an increase in water alkalinity, we found that lampricide uptake in medium (150 mg L^{-1} $CaCO_3$) and high alkalinity waters (250 mg L^{-1} $CaCO_3$) was reduced by 2- to 4- fold when compared to low (60 mg L^{-1} $CaCO_3$) alkalinity waters. In addition, changes in water pH had the largest influence on TFM uptake rates, as increases in pH from 6.5 to 9.0 reduced lampricide uptake rates by 3- to 6-fold. Analysis of ion uptake proteins (Na+/K+-ATPase, vacu-olar (V)-type H+-ATPase) and gill histological preparations will provide information on how the morphology of the gill is impacted by changes in water chemistry and what influence that may have on the sensitivity of sturgeon to TFM.

314 Effects of early-life exposure to PAH-contaminated sediment in fish

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Polycyclic aromatic hydrocarbons (PAHs) are a class of environmental contaminants, which are known to be toxic to early-life stages of fish. Here, we use a combination of laboratory and in situ methods to assess the impacts of early-life exposure to contaminated sediments on bio-chemical (e.g. gene expression, oxidative stress, and DNA methylation), and organismal-level (e.g. mortality and deformities) outcomes. In a laboratory-based study we exposed zebrafish (*Danio rerio*) from ≤ 4 hpf to 120 hpf to sediments collected from a remediated (control) and two PAH-contaminated sites in Lake Saint-Louis, QC. Significant differ-ences in mortality were observed among treatments ($p< 0.0001$); larvae exposed to sediment from one of the PAH-contaminated sites had a higher mortality rate (43%) compared to the remediated site (27%). Furthermore, cytochrome P4501A (CYP1A) expression was induced in individuals exposed to sediment from the two PAH-contaminated sites (30 to 40-fold induction range). Levels of global DNA methylation did not change across treatments and ranged from 86–87%. In addition to the laboratory-based study, we are currently developing an in situ method to assess early-life toxicity to fish in the field. In May 2017, walleye (*Sander vitreus*) eggs were placed in incubator boxes and deposited at the three sites in Lake Saint Louis (the same locations that were targeted in the laboratory study). Larvae were collected after 18-20 days and preserved for future molecular biomarker work (oxidative stress, DNA methylation, gene expression). The use of this in situ method, in conjunction with more controlled in vivo laboratory-based dosing studies, will be beneficial for assessing impacts that early-life exposure to contaminated sediments has on the health of fish.

315 Comparing the photo-induced toxicity of polycyclic aromatic hydrocarbons in two amphibian species

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Aquatic organisms are rarely exposed to only one stressor in their environment. Chemical stressors such as contaminants along with wavelengths of solar radiation can interact to increase toxicity of contami-nants. Polycyclic aromatic hydrocarbons (PAHs) are one class of organic molecules that are lipophilic and readily bioaccumulate. Certain PAHs exhibit photo-induced toxicity with ecologically relevant intensities of ultraviolet (UV) light, resulting in increased mortality, reduced fecundity, and behavioral effects in aquatic organisms. Early life-stages of amphib-ians may be particularly susceptible to PAH photo-induced toxicity as they are translucent, have permeable skin and undergo embryo and larval development in shallow ponds. Limited studies have investigated the

potential photo-induced toxicity of PAHs in larval amphibian species making it difficult to predict their impact. The objective of the present study was to evaluate and compare the sensitivity of a laboratory model amphibian (*Xenopus laevis*) and an ecologically native species (wood frog, *Lithobates sylvaticus*) to the photo-enhanced toxicity of PAHs. 96-h tests were performed in which tadpoles were exposed to individual PAHs (anthracene, naphthalene, benzo(a)pyrene) for 8 h, transferred to clean water, and then exposed to UV light for 12 h. Results were compared using mortality, growth, body burden, and whole body transcriptomic responses as endpoints. In the presence of UV light, anthracene and benzo(a)pyrene were found to increase mortality, and decrease total length, of both larval *Xenopus* and wood frog. Based on mortality data, *Xenopus* was found to be the more sensitive species, suggesting the model organism might be protective of Ranids with regards to the photo-induced toxicity of PAHs. Body burden analyses at 8 h will identify whether differences in uptake of PAHs contributes to the observed species-specific photo-induced toxicity. Transcriptomic analyses are underway in an effort to further understand the mechanism of toxicity of photo-induced PAHs in larval amphibians by examining genome-wide changes in gene expression following exposure to UV light and benzo(a)pyrene. This study demonstrates that UV exacerbates PAH effects in developing amphibians and that photo-induced toxicity of PAHs is species-specific. Overall, it is important to consider UV as an environmental stressor when assessing the toxicity of PAHs to larval amphibians.

316 Dermal Uptake of Organic Contaminants by Amphibians Based on Skin and Contaminant Physicochemical Properties

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Most exposure models and toxicity data available are derived from mammalian and/or avian studies, where the oral route of exposure typically dominates. Such data may not be protective or representative of terrestrial amphibians and thus their incorporation into risk assessment may be difficult. Dermal exposure, which is likely the dominant uptake route for amphibians for most contaminants, is likely dependent on the structural and physiological properties of the exposed skin surface. Further, physicochemical properties likely differentially impact how chemicals move through different areas of the skin. The objective of this study was to characterize both ventral and dorsal dermal uptake of contaminants across a range of moderately to highly hydrophobic chemicals in Blanchard's cricket frogs. Live amphibians were dosed with contaminants (atrazine, lindane, fluoranthene, benzo(b)fluoranthene, and bifenthrin) applied using a dermal patch system on either the ventral or dorsal side. Individuals were collected at specific time points, sacrificed, and prepared for analysis. Excised exposed skin samples and remaining carcass were processed separately using the QuEChERs method and analyzed using GC/MS. Insight into the variability of chemical movement based on the contaminant physicochemical properties, as well as the structural and physiological properties of the exposed skin, should allow risk assessors to better predict doses to these species from measured or predicted environmental concentrations.

317 Toad Prey-Orientation Sensitivity to OP Exposure

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Prey capture in toads consists of a stereotypical series of behaviors beginning with orientation toward the prey item, followed by pursuit and striking. Goals of this study were to characterize prey-orientation behaviors, develop them as a behavioral toxicity assay, and assess their sensitivity to OP exposure. We evaluated different time intervals and presence or absence of a food rewards to establish conditions leading to a consistent number of orientations (51 ± 8/minute). Next, toads were exposed in a preliminary trial to a solvent vehicle (70% DMSO) or Chlorpyrifos oxon (0.4 mg/kg in DMSO) and orientations were assessed daily. Average number of orientations/min decreased by 60% after oxon exposure compared to controls (51 vs 20). DMSO alone had no effect on orientations. Notably, oxon-treated toads showing reduced orientations still ate the provided reward, demonstrating a sustained motivation for food. Further results will be presented and discussed relative to the usefulness of prey-orientation behaviors as indicators of toxicity following exposure to a series of environmentally relevant levels of Chlorpyrifos oxon.

318 Effects of atrazine and selenium on larval and adult aquatic insects

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Aquatic insects link aquatic and terrestrial ecosystems through their metamorphosis and subsequent transition from water to land. Chemical stressors in freshwater, such as agricultural contaminants, have the potential to disrupt insect life cycles and reduce the number of insects emerging as terrestrial adults, thereby damaging or severing this linkage. Atrazine and selenium, though frequently detected in waterways and often co-occurring, have not been studied together in controlled experiments previously. We conducted a mesocosms experiment over six weeks in the summer of 2016 to measure the responses of larval and emerging aquatic insects to treatments of atrazine, selenium, and a combination of the two. Preliminary results indicate that during peak emergence, control treatments had 40-60% higher emergence than that of the contaminant treated tanks, but there were no differences among the atrazine, selenium, and combination treatments. Benthic insects did not respond in the same manner, with all treatments remaining comparable to the control untilthe last week of sampling, when minor reductions in abundance were observed. Our results indicate that atrazine and selenium, alone and in combination, have the potential to impair linkages between aquatic and terrestrial ecosystems through reduction of aquatic insect emergence, and that the effects of these contaminants may differ between benthic and emerging insects.

319 An ecotoxicology approach to researching petroleum spills through mesocosms, field observations and bioassays

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Oil development has expanded dramatically in Colorado over the last decade. Associated with the rapid expansion has been an increase in the number of accidental releases into the environment. Here, we explored a breadth of effects of petroleum spills on coldwater stream communities using data collected from a spill site, as well as data from spills simulated in a laboratory and in a mesocosm facility. First, we analyzed stream health indicators across multiple levels of biological organization to identify long-term ecological impacts associated with a petroleum spill field site. Histological pathologies in mottled sculpin, *Cottus bairdii*, and alterations in benthic macroinvertebrate communities were discovered at the spill location and at downstream locations more than two years after the spill occurred. Subsequently, we conducted two mesocosm experiments, using naturally colonized benthic macroinvertebrate communities. Exposure to simulated spill conditions caused concentration-dependent macroinvertebrate drift and mortality that occurred rapidly after the spills were initiated and at lower concentrations than expected. In addition, concentration-dependent lethal and sub-lethal effects were observed in rainbow trout, *Oncorhynchus mykiss,* subjected to simulated spill bioassays. We conclude that petroleum spills in coldwater streams risk adverse acute, chronic, lethal and sub-lethal effects to aquatic communities. Moreover, by utilizing field observations, mesocosms and bioassays we gained insights into consequences of petroleum spills using an ecotoxicological weight-of-evidence approach.

Adverse Effects of Chemicals on the Microbiome

320 Host-microbiome responses to DEHP exposure: A multigenerational obesity study in danio rerio

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Environmental chemical exposures to obesogens may contribute to the rising obesity epidemic observed across the globe. With more than double the adults in the United States obese or overweight compared to 1980, it is possible that exposures to obesogens are exacerbating the problem. Phthalates, a class of plasticizers used in commercial products such as medical devices, water bottles and personal care products, have become environmentally ubiquitous, and humans and aquatic organisms alike are exposed daily. Phthalates are possible obesogens, but an exact mechanism of action is not yet known. We hypothesize that changes in the gut microbiota coupled with the host responses to such changes in the microbiome may lead to obesity, and that such changes in the microbiome may be heritable from one generation to the next. Due to structural and functional similarities between the human and zebrafish (ZF) guts, ZF were used a model to study the potential heritable effects of diethylhexyl phthalate (DEHP) exposure on the microbiome and any associated that may be related to obesity. The F0 generation was randomly assigned one of three treatments: 1) Control: 5 mg food/fish/day, 2) Overfed: 20 mg food/fish/day and 3) Overfed with DEHP: 20 mg food/fish/day with 3 mg DEHP/kg food. After a 60 day exposure, the ZF in each treatment group were bred and then euthanized so that gut and fecal matter, among others, could be excited for RNA seq analysis and microbiome analysis, respectively. The F1 offspring were exposed as adults for 60 days with the following regime: 1) Control offspring fed 10 mg food/fish/day, 2) and 3) Overfed offspring fed 10 mg food/fish/day or 10 mg food/fish/day with 3 mg DEHP/kg food, and 4) and 5) Overfed with DEHP offspring fed 10 mg food/fish/day or 10 mg food/fish/day with 3 mg DEHP/kg food. Following breading for the F2 generation, the F1 ZF were euthanized in the same manner as the F0 ZF. Previous results from the F0 generation indicated changes in genus level β diversity, increases in Bacteriodetes and decreases in Fusobacteria and Tenericutes phyla in the treatment groups, changes which have been associated with colorectal cancers, obesity and other gut-related diseases. The same microbiome analysis is being conducted on the F1 ZF, and the results will be compared. This data will allow us to test our hypothesis that chronic DEHP exposure leads to heritable alterations in the gut microbiome which could contribute to obesity.

321 Understanding how interactions of single-walled carbon nanotubes with the gastrointestinal system may alter the composition of the gut microbiome

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The efficiency of the multifunctional gastrointestinal system is heavily influenced by the gut microbiome, the community of microbes lining the gastrointestinal tract that plays a role in metabolism, nutrient absorption, and immune function. Despite the versatile role of the gut microbiome in maintaining gastrointestinal homeostasis, the effects of interactions between toxic chemicals and the microbiome are not well understood. Carbon based nanomaterials, especially single-walled carbon nanotubes (SWCNTs), have emerged as a class of contaminants of particular concern due to their high production volume and increased likelihood of environmental release. Due to the hydrophobic behavior of SWCNTs in aquatic environments, these chemicals likely settle onto sediments and organic material, where they may enter the food chain through dietary routes. Recent evidence from our group has found that, while SWCNTs themselves are not physically taken up by the gastrointestinal system, they can induce molecular changes at the gastrointestinal interface, altering the regulation of genes encoding for the peptide transporters, *pept1* and *pept2,* and the satiety hormone cholecystokinin. These findings indicate that SWCNTs can interact with the gastrointestinal environment; however, the specific interactions and their consequences remain unclear. In order to investigate how dietary exposure to SWCNTs may change the gastrointestinal environment, we conducted a 3-month feeding study using largemouth bass (*Micropterus salmoides*), a top level freshwater predator likely to be exposed to SWCNTs through dietary routes. Fish were exposed to 2.5 mg/kg SWCNT-coated food and fed once daily to satiation. At 0, 4, and 11 weeks of exposure, guts were removed, sectioned into proximal, middle, and distal parts, and stored at -80 for analysis. Next-generation sequencing was used to identify differences in the composition of the gut microbiome associated with dietary exposure to SWCNTs, and a combination of analytical, histological, and biomolecular methods to observe SWCNT-induced changes in endpoints such as nutrient absorption, gut inflammation, feeding behavior, membrane permeability, and gastrointestinal lipid composition. Data from this study will increase our understanding of impacts of chemicals on the gastrointestinal environment and help elucidate potential environmental risks associated with exposure to single-walled carbon nanotubes.

322 The response of corals and the coral microbiome to metal exposure

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The mining and production of Ni is increasing in tropical regions. The potential impacts of these activities on the valuable coastal ecosystems are poorly understood. Specifically, there is little information available on the effects of Ni to corals. Scleractinian corals are keystone species for coral reefs forming vital structural habitats that support other species, resulting in habitats with high species richness and diversity. For these reasons, it is important that future research provides data which can inform the sustainable development of Ni operations in tropical regions. This study aimed to investigate the effect of dissolved Ni exposure to the scleractinian coral *Acropora muricata*. Utilising the facilities at the National Sea Simulator (SeaSim), flow through chambers (2.5L) were used to test the effects of Ni and Cu on adult corals and its associated microbiota. Copper was tested alongside Ni to allow for comparisons with past studies. Four replicate chambers were used for; control, 50, 100, 500, 1000, 10000 µg/L Ni and 5, 20, 50, 100 µg/L Cu. Each replicate chamber contained 3 coral fragments (5-8cm in length). After a 96-h exposure, 1 fragment from each chamber was sacrificed for 3 different analytical purposes. One replicate was air blasted to remove tissues which were flash frozen and later used for DNA and RNA sequencing of the microbiota to observe if the bacterial community structure changed in response to metal exposure. A second fragment was air blasted to remove tissues, which were then acid digested and analysed by ICP-MS to determine metal concentrations in the coral tissues. A third replicate was frozen for subsequent metal uptake and distribution analyses using elemental mapping techniques including CT scanning and XRF-ITRAX. Control treatments remained healthy throughout the exposure. After 36 h, bleaching was observed in corals exposed to 50 and 100 µg Cu/L and 10000 µg Ni/L. At 96 h significant discolouration of corals was observed in Ni treatments 500 and 1000 µg Ni/L. The effects of Cu and Ni on adult corals and associated microbiota will be discussed.

323 Zebrafish as a Resource for Characterizing Gut Microbiome-Exposure Interactions

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Mounting evidence indicates that the gut microbiome mediates the effects of environmental exposure on vertebrate health and physiology. However, understanding the mechanisms through which the microbiome modulates exposure has been challenging to determine, in part because of the combinatorial complexity of exposure and the large inter-individual variation in the gut microbiome. Zebrafish may serve as an effective model for discerning these mechanisms due to the large sample sizes it affords as well as an availability of extensive molecular tools. We recently conducted a series of investigations aimed at discerning and advancing the utility of zebrafish as a model for characterizing microbiome-exposure interactions and their health effects. Specifically, we quantified the sensitivity of the zebrafish gut microbiome to various exposures, including consumer grade antibiotics, dietary micronutrients, and environmental toxicants. Additionally, we assembled an integrated gene catalog from metagenomes of the zebrafish gut microbiome, which we used to assess how the functional potential of the gut microbiome varies upon exposure. These data were also used to quantify how the functional diversity of the zebrafish gut microbiome correlates with that of mammalian gut microbiomes. Collectively, our investigations underscore the utility of zebrafish as a means of characterizing the effect of environmental chemical exposure on the gut microbiome.

324 Gut Dysbiosis in Animals Due to Environmental Chemical Exposures

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The gut microbiome consists of over 1×10^3 to 1×10^4 distinct microbial inhabitants. Together they possess 150 times more genes (the microbiome) than the human genome and thus, the gut microbiome should be considered an "organ" in and of itself. Gut microbial communities are dynamic and susceptible to changes in the host environment and body condition. In turn, there are consequences for the host due to disturbances in the gut microbiome. Gut dysbiosis might result in obesity, diabetes, gastrointestinal, immunological, and neurobehavioral disorders. These diseases can originate due to shifts in microbiota favoring more pathogenic species that produce various virulence factors, such as lipopolysaccharide. It has been shown that gut bacteria may be transmitted to distal target sites, including the brain. Other potential mechanisms by which gut dysbiosis can affect the host include bacterial-produced metabolites, production of hormones and factors that mimic those produced by the host, and epimutations. All animals, including humans, are exposed daily to various Environmental Chemicals (ECs) that can alter the delicate balance of the gut microbiome and may lead to downstream systemic effects secondary to these gut microbiome disturbances. Increasing reports have shown that EC exposures can target both host and the resident gut microbiome. The current knowledge of how endocrine disrupting chemicals (EDCs)- in particular bisphenol A and ethinyl estradiol, heavy metals, air pollution and nanoparticles can influence the gut microbiome and subsequently influence pathophysiological responses in the host will be discussed. Understanding pathology of EC-induced gut dysbiosis may identify new remediation strategies in animals, including humans, exposed to such compounds.

325 Engineered nanomaterials alter the gut microbiome of freshwater snails through chronic exposure in outdoor wetland mesocosms

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Wetlands are frequently exposed to emerging contaminants through wastewater and other anthropogenic sources. Engineered nanomaterials are increasingly popular compounds in consumer products and are used in some pesticides to manage agricultural pests and diseases including microorganisms. Snails are dominant grazers in many freshwater ecosystems and understanding if emerging contaminants alter gut microbiome function could have ecosystem wide implications. We examined the effects of Kocide 3000, an agricultural fungicide/bactericide composed of copper hydroxide engineered nanoparticles, and citrate-coated gold nanoparticles on the gut microbiome of a ubiquitous and numerically dominant freshwater snail (*Physella* sp.) after nine months of chronic exposure in replicated, outdoor wetland mesocosms. The digestive tracts from multiple snails per mesocosm were aggregated and DNA was extracted. Subsequently, the V4-V5 region of 16S rRNA gene was sequenced using an Illumina MiSeq and processed with QIIME. Preliminary analysis suggests that Kocide and gold treated snails have a higher proportion of *Firmicutes*, specifically relative to controls. Overall, *Firmicutes* was the most abundant phylum, followed by *Proteobacteria*, *Actinobacteria*, *Cyanobacteria* and *Planctomycetes*. There were significant changes in the gut microbiome that could have implications for snail growth, food assimilation, and nutrient recycling after long term exposure to a bactericidal nanomaterial.

326 Comparing Acute High Dose and Chronic Low Dose Exposures to Acid Mine Drainage on Sediment Microbiome Communities in the Animas River Watershed

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Acid mine drainage is potent mixture of environmental toxicants with extremely low pH and high concentrations of several toxic metals. Unfortunately, it is constantly seeping from an estimated 500,000 active and abandoned mines across the United States. Near the headwaters of the Animas River exists a large concentration of hard rock mines. These mines release acid mine waste that infiltrates the Animas watershed, exposing downstream sediment microbiomes to increased toxicant loads and extremely low pH conditions. The aim of this study is to compare two different types of exposures to acid mine drainage, long-term low dose exposures and a short-term high dose in combination with the long-term exposure. Sediment along the Animas River before the confluence with Cement Creek has been exposed to acid mine drainage since the late 1800s, when the mining industry first started in Southern Colorado, creating low dose constant exposure to acid mine drainage. On August 5, 2015 the Gold King Mine, located just above Cement Creek, was breached releasing an estimated 3 million gallons of acid mine drainage, creating the short-term high dose scenario. We collected sediment from the Animas River Watershed in early January 2016, about five months after the spill. To analyze the effect of these different exposure scenarios we conducted amplicon sequencing of the 16S, 18S and ITS genes to identify sediment microbial community members. Additionally, we took environmental measurements and analyzed sediment samples by ICP-OES to quantify the metal concentrations. We observed obvious shifts in community structure between the different exposure scenarios downstream of acid mine drainage. The 16S analysis showed that in the lowest pH and highest metal concentrations seen in Cement Creek the most abundant microbe was *Gallionella sp.,* an iron-oxidizing species of bacteria that could contribute to the natural remediation of acid mine drainage. The long-term, low-dose exposure scenario had increased alpha diversity when compared to the short-term high dose exposures. Additionally, low-dose exposures showed community structures more similar to less impacted, downstream communities. Future work will focus on the shifts in gene abundance in each of the exposure scenarios as well as downstream of the mines releasing acid mine drainage.

327 Toxicity of Military Relevant Chemical Contaminants on a Reptilian Model Species' Microbiome

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Reptiles make excellent contaminant indicator species because they are constantly sampling their environment, feeding on organisms that may have bioaccumulated a contaminant or through direct ingestion of a contaminant through low levels of geophagy. The green anole has been developed as a reptile model for toxicity studies, including recent work on toxicity of select explosives. Few comprehensive toxicity datasets exist with military compounds, and this project aims develop those data to complement the characterization of the microbiome. The legacy military contaminants 2,4,6-trinitrotoulene (TNT) and lead (Pb) as well as the emerging military contaminant, 2,4-dinitroanisole (DNAN) were selected as test contaminants to address the notion of whether or not the lizard gut microbiome can serve as a predictive biomarker for contaminant exposure. Initial data preceded exposures and revealed lizard microbiome microbial diversity decreased in individual wild-caught lizards following acclimation with a consistent diet. Microbiome composition comparisons across lizards indicated that acclimated lizard microbiomes formed a distinct cluster compared to their initial "wild" microbiome signatures. Once acclimated, cricket fed lizards presented a stable microbial community profile and the effects of alternating diets from crickets to mealworms did not significant alter lizard microbiome signatures. The role of contaminant (TNT, Pb, DNAN) exposure in mediating these patterns will be presented.

Ecosystem Services as a Basis for Ecological Risk Assessment of Chemicals – New Approaches and Research Needs

328 Chemicals: Assessment of Risks to Ecosystem Services (CARES). Where are we and where are we going?

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Chemicals may have positive or negative effects on human well-being. The challenge facing decision makers is how to balance the wellbeing benefits provided by the use of chemicals with the potential wellbeing costs via habitat degradation and loss of ecosystem services. Assessing the risk of chemicals to ecosystem services needs the development of tools and approaches for (1) identifying what needs to be protected where and (2) translating ecotoxicological exposure and effects information into risks for ecosystem service delivery. The CARES project brought together stakeholders from government, business and academia to develop a common understanding of the merits and feasibility of an ecosystem services approach to chemical risk assessment and the implications for implementation. The project was organised in three phases. Phase 1 assessed the current state of knowledge and identified key information gaps and challenges (e.g. complexity in assessment, data requirements, limitations in current testing/assessment methods). The need for a tiered approach was identified: lower tier using exposure- and/or effect-based triggers based on conservative assumptions; higher tier using standard scenarios to account for temporal and spatial heterogeneity in use and exposure patterns, ecological communities and ecosystem functions. Phase 2 explored the use of novel approaches from ecology, ecotoxicology and ecological modelling to address key information gaps. Case studies were used to illustrate the development of environmental scenarios and the potential use of ecological models and trait-based approaches to address issues of ecological complexity and heterogeneity. Stakeholders identified and prioritized research needed to effectively implement an ecosystem services approach into prospective and retrospective risk assessment. The top four needs were: linking measurement endpoints to ecosystems services; mechanistic models, in particular ecological production functions; scenario development; integrated decision making framework for risk managers and risk assessors. Phase 3 explored how an ecosystem services approach could be implemented and considered the implications for regulatory risk assessment.

329 Aligning ecological models and ecosystem service endpoints

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A challenge in the integration of ecosystem services (ES) into ecological risk assessment is finding ecological models with endpoints that align with ES. EPA's EcoService Models Library (ESML) is an online database of ecological production functions (EPFs), or models useful for estimating the production of ecosystem services. ESML, currently available as a beta release, describes >125 EPFs or EPF applications. It shows how each EPF aligns with ES under two classification systems: USEPA's National Ecosystem Services Classification System and the European Environment Agency's Common International Classification of Ecosystem Services. This presentation discusses which classes of ES are covered, or not covered, by this population of EPFs, and the implications for ecological risk assessment.

330 Predicting impacts of an endocrine disruptor on ecosystem services provided by fish populations

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In this presentation we demonstrate how a mechanistic modeling approach can be used to predict whether and how toxic responses to chemicals at (sub)organismal levels in model species (i.e., what we typically measure) translate into impacts on ecosystem service delivery (i.e., what we care about). We consider a hypothetical case study in which two species of trout, brown trout (*Salmo trutta*) and greenback cutthroat trout (*Oncorhynchus clarkii stomias*), living in a high-altitude river system are exposed to human-derived estrogen (17a-ethinylestradiol, EE2), which is the bioactive estrogen in many contraceptives. We use the individual-based model, inSTREAM, developed by Railsback and colleagues, to explore how seasonally varying concentrations of EE2 on male sperm quality influence recruitment of the two species and the consequences of such for recreational ecosystem services as well as non-use services (since the cutthroat is the state fish of Colorado though nearly extinct). The inSTREAM model incorporates seasonally varying river flow and temperature, fishing pressure, species-specific life-history, and inter-specific competition together with impacts of EE2. These mechanisms lead to complex and sometimes counterintuitive predictions of contaminant effects. For example, EE2 may reduce brown trout, but be neutral to cutthroat trout because its direct effects are offset by reduced competition from brown trout. We discuss some of the advantages and challenges of using this kind of approach and outline the research needed to move it forward.

331 Linking organism-level effects of chemical stressors to effects on ecosystem services: A case study of a chemically impacted reservoir

C.J. Salice, Towson University / Biological Sciences/ Environmental Science; R. Bruins, USEPA / Ecological Exposure Research Division; V. Forbes, N. Galic, University of Minnesota / Ecology, Evolution, Behavior; R.A. Pastorok, Integral Consulting, Inc.; A. Kanarek, USEPA / Office of Pesticide Programs; B. Birnir, University of California, Santa Barbara; V. Ducrot, Bayer CropScience AG / Environmental Safety Ecotoxicology; K.V. Garber, USEPA / Office of Pesticide Programs Environmental Fate and Effects Division; H. Jager, Oak Ridge National Laboratory; S. Railsback, Lang Railsback & Associates; R. Rebarber, University of Nebraska, Lincoln; P. Thorbek, Syngenta / Environmetal Safety

Although protection goals for the ecological risk assessment of chemicals are increasingly being framed in terms of ecosystem service delivery, the type of data collected to assess risk is generally at the level of individual organisms. Currently, extrapolation from what is measured to what we want to protect uses overly simplistic approaches (e.g., hazard or risk quotients) that are not solidly grounded in biology or mathematics. This presentation describes progress and ongoing efforts from a working group funded by the National Institute of Mathematical and Biological Synthesis (NIMBioS) to develop a systems modeling framework to link organismal effects to changes in ecosystem services. The proposed framework quantitatively links physiological energetics, to organism performance, population dynamics, and ecosystem service delivery. Here we present a case study involving the impact to ecosystem services in a reservoir that receives pesticide input. The AQUATOX model was used to explore impacts of a model organophosphate pesticide on several ecosystem services including water clarity (measured as secchi depth) and fishability (measured as game species biomass). Both ecosystem services are important for recreation, and both are impacted by zooplankton for which *Daphnia magna* is the representative species and highly sensitive to the model pesticide. Results show an exposure-dependent response to the pesticide but also a considerable amount of natural variability. We are exploring metrics such as the number of days during the recreational season that are negatively impacted by pesticide exposure as a means of linking the ecosystem effects to impacts on services. Importantly, the efforts reported here are designed to dovetail with an additional, coordinated NIMBioS working group focused on a modeling framework that links suborganismal (metabolic, cellular, and molecular) responses to chemicals to effects that manifest at the organism level. The overarching goal of these collaborative, multidisciplinary groups is a generalizable modeling framework and proof of concept that can be used to relate toxicant responses at levels of organization that are more easily studied (sub-organismal, organismal) to outcomes relevant to society and effective environmental management (ecosystem services).

332 Using Influence Diagrams to Map Adverse Outcome Pathways from Molecular Initiating Events to Ecosystem Services

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Considering ecological risk assessment outcomes in terms of ecosystem services – the benefits derived by humans from the natural environment – requires greater analytical complexity than the chemical-by-chemical approach utilized currently. Coupled human-natural systems, which is what ecosystem services explicitly address, includes exposures to chemicals in the context of other stressors and outcomes. This increased complexity, while more realistic, poses methodological challenges. We are exploring one approach involving the use of a Bayesian Network or influence diagram as a framework for considering multiple influences on ecosystem services related to salmon populations in the Pacific Northwest. A second application involves exposure to perfluorinated compounds and reproductive outcomes in birds. Ecosystem services related to birds include their roles as predators, pollinators, scavengers, seed dispersers, seed predators, and ecosystem engineers. The Bayesian network maps pathways of influence from exposures and stressors through key events and relationships to outcomes of regulatory interest. The toxicity portion of the network is based on an adverse outcome pathway (AOP) approach that better integrates both in vitro and in vivo evidence to provide a more mechanistic based understanding of the implications of exposure to contaminants. Mapping pathways and key events provides an operational framework for including the influence of other potentially non-chemical stressors on key events (e.g., dissolved oxygen or habitat quality). Bayesian mathematics is based on conditional probabilities, and once the influence diagram is parameterized (which can involve the use of underlying process models and other kinds of models), the framework allows greater flexibility in identifying opportunities for management interventions. Outcomes are typically defined in terms of populations, requiring the use of population models. We will provide an overview of our work to date funded through a Science to Achieve Results grant and will highlight both key challenges as well as opportunities for improving risk-based decision-making in the context of ecosystem services.

333 Food webs as collections of traits and agents: Model development and application to multiple stressors

N. Galic, V. Forbes, University of Minnesota / Ecology, Evolution & Behavior

Freshwater ecosystems have been exposed to profound changes caused by urbanization and agriculture. Agricultural intensification has caused increased input of nutrients and toxicants into water bodies adjacent to agricultural fields often adversely impacting non-target biota, ecosystem processes, and the delivery of ecosystem services. Food webs are at the core of freshwater ecosystems and provide many ecosystem services, such as presence of game fish populations and protected species, aesthetical appeal and clean water. Impacts of multiple stressors are often investigated in isolation, but they often co-occur in aquatic ecosystems, with potentially detrimental consequences for structural and functional ecosystem properties, including food web dynamics. To produce scientifically underpinned management to ensure persistence of ecological systems, we need approaches that are predictive and robust. Modeling approaches offer the possibility to test many stressors – in isolation and in combination – on realistically constructed food webs and on various spatial and temporal scales. Approaches that focus on traits, rather than on species, as structural components of ecosystems have the ability to make predictions that go beyond specific systems and are not geographically constrained. Here we present development of a stream food web model and its application to multiple stressors. Species in the food web are not represented explicitly, but as agents with combinations of traits defining their functional guild. More specifically, primary consumers are differentiated into herbivores, detritivores and predators, and secondary consumers into invertivores, piscivores, and omnivores. Agents' life history emerges from the rules of Dynamic Energy Budget theory, which defines their metabolic traits through maximum body sizes observed for respective trophic guilds. The food web also includes three resource pools: autotrophs, detritus and nutrients. We briefly describe the model and how well it represents food webs in forested streams. We then describe a model application to a study aiming to quantify impacts of multiple stressors on several food web endpoints, such as persistence and biomass proportion of different functional groups and the stability of food web compartments. We specifically looked at how stressors which have bottom-up effects and those that have top-down effects interact and impact food web properties and the services they deliver.

334 Ecological Production Functions Linking Multiple Stressors to Ecosystem Services - A Case Study

W.R. Munns, A. Kuhn, M. Mazzotta, D.E. Nacci, USEPA / Atlantic Ecology Division

The ecosystem services concept is being used to frame environmental protection goals that guide management of the risks of chemicals. Ecosystem services link changes in ecological systems to the benefits received by people. The use of ecosystem services in risk assessments and the environmental protection decisions they support calls for an understanding of the effects of human-mediated stressors on ecological production functions (EPFs), defined as the types, quantities, and interactions of natural features required to generate ecosystem services. Here we describe an EPF that links co-occurring human-mediated stressors to the dynamics of Common Loon (*Gavia immer*) nesting in New Hampshire lakes to ecological benefits, such as wildlife viewing, which can be estimated from housing prices on lakes with and without loons. The occurrence and success of nesting loons are affected by diverse and interacting stressors operating at different spatial scales, i.e., toxic mercury bioaccumulated in loon eggs (through the consumption of atmospherically-deposited mercury in lake prey fish), water quality declines related to nutrient runoff from increasing shoreline development, and direct human disturbance of nests. This case study highlights the ecological complexity that can be inherent to EPFs and their use when assessing risks to ecosystem services of chemicals in the context of other stressors.

335 Ecosystem Services Evaluations for Superfund Cleanups

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Ecosystem services (ES) communication concepts and quantification tools may potentially link a contaminated site's ecological risk assessment (ERA) to its greener cleanup strategy and anticipated ecological reuse. To explore how ES quantification may occur at contaminated sites, two Superfund cleanup sites, of different sizes and in different ecological regions, were selected for ES evaluations. The Lower Darby Creek Area includes former landfills, now covered in forest and wetland habitat, in metropolitan Philadelphia, whereas the Lower Basin of the Coeur d'Alene River is 18,000 acres of mining-impacted wetlands in rural northern Idaho. Evaluation tools need to assist in (1) identification of site-specific ES, (2) quantification of ES endpoints, (3) assessment and prediction of ES pre- and post- remedy operations respectively, and (4) identification and implementation of greener cleanup best practices that minimize impact to ES or improve ES. The ERA, Record of Decision (ROD), Reuse Assessment, and discussion with the project managers provided information for identification of ES at each site. To quantify ES, publicly available tools were reviewed. Five tools (Final Ecosystem Goods and Services Classification System (FEGS-CS) Query Tool, EnviroAtlas, InVEST, i-Tree Eco, and the Wetland Ecosystem Services Protocol (WESP)) were trialed to determine their utility for different site scales, ecosystem types, and remediation scenarios. Though selection of an ES tool(s) at a particular site will be dictated by the landscape setting and environmental characteristics at that site, our ultimate goal is to develop a methodology or protocols for ES evaluation that can be applied over a wide range of Superfund sites.

Advancing the Adverse Outcome Pathway Concept – An International Horizon Scanning Approach

336 Advancing the Adverse Outcome Pathway Framework - An International Horizon Scanning Approach

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In preparation for a Society of Environmental Toxicology and Chemistry Pellston Workshop titled "Advancing the Adverse Outcome Pathway (AOP) Concept: An International Horizon Scanning Approach," a horizon scanning effort was undertaken to gather input from the global scientific and regulatory communities regarding the perceived state of the science regarding AOP development and application. The horizon scanning exercise specifically solicited questions concerning challenges and/or limitations that must be overcome to move the AOP framework forward. From March-June, 2016, 340 valid questions were collected from 158 global submissions to an online horizon scanning survey, with respondents self-identifying as 35% academia, 35% government, 20% industry, and 5% non-government organizations. The questions were separated into broad topic areas including, AOP networks, quantitative AOPs, collaboration and communication on AOPs, AOP discovery and development, extrapolation, exposure/toxicokinetics considerations, and AOP application. An expert-ranking exercise was then conducted to identify high-priority questions for each category and from this, four key themes emerged that could aid in guiding future AOP research and regulatory initiatives. These themes were therefore used as workgroup topics for the Pellston Workshop, including: (1) AOP networks and their applications, (2) quantitative AOPs (qAOPs) and their applications, (3) regulatory use of the AOP framework, and (4) expanding awareness of, involvement in, and acceptance of AOPs to support aspects of predictive toxicology and regulatory decision-making. Additionally, from the horizon scanning exercise, frequently asked questions (FAQs) were identified and addressed by experts in the field. The answers to these FAQs can provide a common starting point for those interested in the AOP framework to initiate discussions around new, forward-thinking approaches. Together the horizon scanning approach, expert ranking exercise, and answers to FAQs, were used to set the stage for the SETAC Pellston Workshop that took place in Cornwall, Canada during April 2017. The contents of this presentation neither constitute nor necessarily reflect USEPA policy.

337 Adverse Outcome Pathways: Moving from a scientific concept to a globally accepted framework

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The adverse outcome pathway (AOP) framework has gained significant international traction as a systematic approach for capturing existing knowledge to transparently link mechanistic data to apical toxicity endpoints as a means to inform research and risk assessment. While the framework has evolved significantly since its introduction in 2010, it was recognized that a survey of the broader scientific community would be useful in identifying shortcomings and in guiding future initiatives. In 2016, we reached out to national and international scientific and regulatory communities to collect questions and provide an opportunity to discuss key outstanding challenges that must be addressed in order to realize the full potential of the AOP framework. Four key themes emerged from this “Horizon Scanning” exercise (see presentation “Advancing the Adverse Outcome Pathway Framework - An International Horizon Scanning Approach” in this session), which were then addressed at a Society of Environmental Toxicology & Chemistry (SETAC) Pellston™ Workshop comprised of international participants representing industry, government, academia, and NGOs was held in Cornwall, Ontario, in April 2017. This presentation will provide an overview of the overall outcomes and common themes that emerged during this Pellston Workshop. In brief, common themes that spanned across these main topics included the need to simplify, translate, and better communicate the AOP framework to the broader international stakeholder community, and a consensus that the AOP framework does not represent a rigid tool but rather a knowledge repository for diverse stakeholders ranging from epidemiologists to mainstream experimental toxicologist to risk assessors and managers. Furthermore, when considering the AOP framework and its applications, the field of environmental toxicology and human health naturally merged into a continuum that is at the nexus of Toxicology in the 21st century. In particular, it was felt that the current momentum the AOP framework has gained across a wide range of professional sectors provides the unique window of opportunity to reach out to and gain acceptance of this framework by society, which will be required for it to become an integral part of the international chemical and environmental risk assessment landscape. The contents of this presentation neither constitute nor necessarily reflect USEPA policy.

338 Adverse Outcome Pathway Networks: Development, Analytics, and Applications

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The adverse outcome pathway (AOP) framework was developed with a recognition that individual AOPs are a pragmatic unit for development and evaluation, but that application of AOPs in real-world exposure and assessment scenarios requires the ability to consider both interactions among multiple AOPs as well as convergent and/or divergent effects of chemical exposures depending on target organ, life-stage, taxa, etc. considered. AOP networks, defined as an assembly of two or more AOPs that share one or more key events, address these more multi-faceted applications of the framework. This presentation briefly outlines critical concepts concerning the development/derivation of AOP networks, how they may be analyzed, and illustrates how information derived from them can be applied. Network development focuses on querying and filtering the AOP knowledgebase to extract an AOP network appropriate for a given research question or problem formulation. Once an AOP network is derived, analytics based on graph theory and/or data layers superimposed on the network diagram may be applied to identify important features or attributes. These and other considerations can aid in the identification of critical pathways through the network that may either dominate observed empirical outcomes or may be most important to a given assessment or application. These concepts are illustrated through a number of application case studies, additionally recommendations for ways to further enhance the utility of AOP networks are provided. The contents of this presentation neither constitute nor necessarily reflect USEPA policy.

339 Adverse Outcome Pathway Network Analyses: Techniques and Benchmarking the AOPwiki

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As the community of toxicological researchers, risk assessors, and risk managers adopt the adverse outcome pathway (AOP) paradigm for organizing toxicological knowledge, the number and diversity of adverse outcome pathways and AOP networks are continuing to grow. This growth includes the description of new AOPs as well as refinement and linking of existing AOPs. This presentation introduces a suite of network analytic and graph theoretic techniques that are relevant for analyzing AOP networks; these techniques are described and displayed through an analysis of the *Collaborative Adverse Outcome Pathway Wiki* (AOPwiki) knowledge base (*AOPwiki.org*). The AOPwiki is a repository for storing and sharing AOPs within the expanding AOP community. As a result of using the AOPwiki to illustrate techniques, this research also serves as a benchmarking effort for the on-going development of the AOP knowledge base. This benchmarking will be useful for understanding the current state of AOP knowledge within the AOPwiki, identifying major points of connectivity among current AOP descriptions, and identifying parts of the overall network that would benefit from further elaboration. The analyses highlighted provide a baseline that will be important for understanding how AOP knowledge is expanding and changing in coming years and how effectively the crowd-sourced model of AOP development is working in practice. The contents of this abstract neither constitute, nor necessarily reflect, USEPA policy.

340 How to Build and Apply Quantitative Models from Adverse Outcome Pathways

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Quantitative prediction and assessment of chemical impacts on human and environmental health is important in understanding the potential hazards and risks of using, or being exposed to, chemicals. Here we examine how the Adverse Outcome Pathway (AOP) concept and knowledge base can be used to develop semi-quantitative and quantitative models (qAOPs) to assess and predict hazards and risks of chemicals. Quantitative models can be developed with a clear problem definition and using AOPs as initial conceptual models. Modeling methods range from semi-quantitative to quantitative modeling approaches or combination of these (e.g. fully mechanistic mathematical /ordinary differential equation based, phenomenological, individual-based models, statistical, or Bayesian network models). We discuss best practices for choosing modeling approaches, model building and the necessity for transparent and comprehensive documentation in order to gain confidence in the use of a model. Finally, we present examples of how qAOP models can support decision making: a screening level assessment of the health hazards of chemicals and chemical mixtures using a qAOP Bayesian network model of steatosis, the use of qAOPs in determining the hazard or risks of a chemical when toxicological data must be extrapolated from a model species or life stage (e.g. in vitro to in vivo extrapolation or IVIVE), and use of qAOPs in a risk assessment context where exposure and toxicokinetics models must be included.

341 Using Adverse Outcome Pathways to Guide Chemical Decision Makers

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An invited group of scientists participated in a SETAC Pellston Workshop^{TM}, "Advancing the Adverse Outcome Pathway (AOP) Concept – An International Horizon Scanning Approach," in April, 2017. This workshop addressed key challenges and limitations to using AOPs as tools for informing regulatory decisions as identified in responses to a Global Horizon Scanning survey. This presentation will summarize the findings of the work group that focused on the use of adverse outcome pathways in chemical decision making. The use of AOPs and related concepts have increased in scientific and regulatory sectors over the past decade, coinciding with pressures to find innovative solutions for evaluating chemical safety in a more efficient and effective manner. This workgroup focused on using AOPs as a tool for the assessment of chemicals at various points across the "life" of a chemical; from research and development within the commercial sector, government registration and regulation, through to post-marketing use and stewardship. Examples are provided regarding how AOPs can be and are currently being used in chemical decision making. Considerations for evaluating the suitability of AOPs, recognizing that the acceptable level of uncertainty varies based the nature of the decision and the context in which the AOP is being applied. The presentation provides multiple examples of AOP use and practical considerations for evaluating whether use of AOPs are fit-for-purpose in different circumstances.

342 Realizing the Promise of AOPs: A Stakeholder-Driven Roadmap to the Future

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The adverse outcome pathway (AOP) framework was developed to serve as a knowledge assembly and communication tool to facilitate translation of mechanistic (e.g., molecular, biochemical, histological) data into adverse apical outcomes meaningful to chemical risk assessment. Although initially designed for ecotoxicology applications, the framework has also received extensive attention relative to chemical safety assessments for human health. Moreover, as the AOP concept and associated knowledgebases have evolved, it has become recognized that the potential stakeholder community is broader than scientists and regulators directly involved in chemical safety assessment. For example, the application of AOP-based thinking for addressing biomedical challenges has become increasingly evident. This presentation will identify various stakeholders who currently, or could potentially, benefit from application of the AOP framework and knowledge to specific needs, and describes collaboration strategies to effectively engage these stakeholders, including how to maintain a viable, sustainable network to support AOP stakeholders. We make recommendations as to how to address the governance and coordination of AOP development and knowledge dissemination in a multi-stakeholder consortium, as well as what the requirements are for ensuring stable financial resourcing for key infrastructure. The contents of this abstract neither constitute, nor necessarily reflect, official USEPA policy.

Integrated Tools for Improving Environmental Fate and Risk Assessment for Unregulated Contaminants and Their Mixtures – Part 2

343 Assessing sources and fate of organic pollutants in San Francisco Bay using non-targeted analysis coupled with discrete and passive sampling

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Organic pollutants enter urban estuaries through a variety of routes, including stormwater runoff, municipal and industrial wastewater, and agricultural activities. The Regional Monitoring Program for Water Quality in San Francisco Bay (RMP) has been engaged in periodic monitoring to assess trends in occurrence of legacy and emerging organic and inorganic contaminants within the Bay. However, there remains much uncertainty regarding the identity and fate of the most relevant emerging contaminants in San Francisco Bay water and sediments, along with their source. This lack of data is most acute for unregulated contaminants, for which there are often no standard methods for analysis. Under the framework of the RMP, we have conducted a comprehensive, non-targeted analysis of polar and semi-polar organic pollutants in San Francisco Bay water and sediment using high resolution/accurate mass (HR/AM) mass spectrometry approaches coupled with computational mass spectrometry and cheminformatics tools. A combination of discrete grab sampling and passive sampling (Polar Organic Chemical Integrative Samplers: POCIS) strategies were employed to collect water-soluble pollutants at sites within the San Francisco Bay watershed thought to be influenced by different contaminant pathways: urban stormwater runoff (San Leandro Bay), agricultural runoff (Napa River), and wastewater effluent (Lower South Bay). 24-hour composite samples of wastewater effluent were also collected from several wastewater dischargers. Finally, surficial sediment samples were taken from associated sites to assess contaminant deposition. Samples were analyzed using HR/AM Orbitrap liquid chromatography high resolution mass spectrometry and resulting data were analyzed using a custom workflow designed for high-fidelity componentization, prioritization, identification, and semi-quantitation of detected molecular features. Compounds identified through this method were reflective of diverse pollution sources, including polymer additives, vulcanization accelerants (stormwater), pharmaceuticals and detergents (wastewater), and herbicides, insecticides, and fungicides (agriculture). Implications of pollutant discharge and accumulation in the San Francisco Bay will be discussed in context of risk to the aquatic ecosystem.

344 Integrating targeted analysis and broad-scope screening at the sub-sewershed scale to understand down-the-drain transport of pesticides

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Pesticides, including pyrethroids, fipronil, and imidacloprid, have been reported in wastewater effluent at concentrations that exceed aquatic toxicity thresholds. In California, discharge of treated effluent can dominate flow in streams and rivers and can contribute to estuarine environments with limited hydrodynamic exchange with the ocean, posing a potential risk to aquatic organisms. Quantitative results at the sub-sewershed scale are necessary to gain an understanding of relative source contributions (i.e., residential, industrial, commercial, institutional, and municipal sources). The usefulness of monitoring results can be greatly enhanced when integrated with broad spectrum high resolution mass spectrometry to chemical fingerprint for relative source contributions. This study examines 24-h time-weighted composite samples (influent, effluent, and ten sub-sewershed sites) collected monthly from February 2016 through January of 2017. Additional samples were collected from sites with potential for relatively large mass flux of pesticides (i.e., pet grooming operation, pest control operator, and laundromat). A target list of pesticides were quantitated in all samples in conjunction with both liquid and gas chromatography time-of-flight mass spectrometry (LC-QTOF-MS and GC-QTOF-MS) to comprehensively screen for roughly 3500 suspect chemicals. LC-QTOF-MS data were acquired using electrospray ionization (ESI) in positive and negative modes with All-Ions fragmentation, and two accurate mass MS/MS libraries containing >2000 chemicals were screened. The extracts were run on GC-QTOF-MS in electron impact (EI) mode using a retention time locked method and the spectra were screened against a pesticide library containing exact mass fragments and retention times. The results of this study provide a comprehensive view of pesticide, including pesticide adjuvant, and other trace organic compound occurrence at the sub-sewershed scale and offer the opportunity to quantify, within a single sewer catchment, the relative contribution sources.

345 Detection and computational screening of biological receptor activity for novel environmental contaminants and transformation products

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Human activities result in the transport of chemical contaminants, toxicants, and other bioactive chemicals to receiving waters, thus contributing to poor water quality and potential adverse outcomes for exposed organisms. To protect ecosystem and human health, identification of high risk, high priority contaminants and toxicants in urban waters is a necessary but challenging analytical task. Here, we present the use of high resolution quadrupole time of flight mass spectrometry to detect novel trace organic contaminants and also explore the formation of transformation products of known high potency pharmaceuticals and drugs in laboratory scale reactors and environmental systems. Following their detection, in silico computational screening of detected, or suspected, compounds and select transformation products was conducted using the Pocketome", a computational library of over 2000 protein binding sites that represent drug/toxicant targets. By combining screening for potential biological interactions, the first indication of possible adverse effects, we can prioritize our environmental occurrence efforts toward assessing the occurrence and environmental fate of those urban environmental contaminants that are most likely to affect ecosystem health. Examples of this screening workflow and select examples of high interest research outcomes will be provided.

346 Developing new organic chemical tracers for hydraulic fracturing wastewaters

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Drilling and hydraulic fracturing of unconventional oil and gas resources involves the use, transport, and disposal of large volumes of fracturing fluids. During any of these processes, fluids may accidentally be released and enter surface waters or groundwater. Hydraulic fracturing fluids, flowback and produced waters [13 sites, Appalachian basin], and four years of stream water baseflow samples [Garrett County, Maryland] were collected and analyzed using electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS). Stream water dissolved organic matter fingerprints were compared to hydraulic fracturing fluid and wastewater mass spectra to find compounds absent from natural waters but present in hydraulic fracturing wastewaters and identify possible tracers. More than 28,000 exact *m/z* ions were identified in hydraulic fracturing wastewaters but were absent from stream water samples, many of which could be unambiguously assigned molecular formulas. These assigned formulas included iodinated compounds, known surfactants, and a number of formulas with low double bond equivalents that may be transformation products of surfactants. Compounds that were present at high intensity and in more than two-thirds of the hydraulic fracturing samples are of particular promise and are being further investigated to determine their suitability as tracers.

347 Evaluating the o-DGT passive sampler as a screening tool for suspect pharmaceuticals in wastewaters using High Resolution Mass Spectrometry

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Our previous work successfully developed and applied the organic - diffusive gradients in thin films (o-DGT) sampler as a quantitative measurement tool for targeted polar organic contaminants in impacted aquatic systems. The present work highlights the o-DGT passive sampler as a useful screening tool for candidate wastewater contaminants based on accurate mass spectrometry data. Since current polar passives (e.g., POCIS) require measured compound-specific sampling rates to back out water concentrations, screening for emerging or understudied chemicals becomes difficult. The simple uptake model developed previously for o-DGT gets around this issue, as demonstrated in the current study. Samplers were deployed at two wastewater treatment plants in the Greater Toronto Area and analyzed with a Waters Xevo G2-XS Q-TOF HRMS. Samples were mined using exact mass data from the literature and qualitative structure confirmation was based on chemical composition software (mass error < 5ppm) and observed versus predicted isotope patterns. Likely structures were elucidated and uptake rates in o-DGT were estimated based on modelled diffusion coefficients, providing semi-quantitative concentration estimates. Venlafaxine, desvenlafaxine, lamotrigine, and ketamine are examples of wastewater contaminants picked up by the screening method. Two surrogate standard approaches utilizing our routine targeted analyte list was used to quantify the suspect analytes based on: 1) individual surrogate responses, and 2) averaged surrogate responses. Suspect concentrations were confirmed using isotope dilution. Our data suggests that quantifying suspect contaminants using the averaged response approach provides a semi-quantitative concentration estimate (within a factor of two) and removes much of the uncertainty around choosing a single surrogate to match the behaviour and sensitivity of the suspect chemical. This work offers evidence for a simple, semi-quantitative approach to suspect screening with passive sampling using high resolution mass spectrometry.

348 Unregulated disinfection by-products: Integration of temporal trends in occurrence, in vitro toxicity, and natural organic matter precursors

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Halogenated disinfection by-products (DBPs) are a diverse class of compounds formed during the treatment of drinking water through the reaction of natural organic matter (NOM) and halogen salts with disinfectants. While Health Canada regulates a handful of DBPs, there are hundreds of novel and unregulated DBPs that have been reported and even more that remain unknown. To expand the library of unregulated halogenated DBPs, we adopted a recently described ultra-high resolution mass spectrometry (MS) method and chemometric strategies to screen chlorinated drinking water collected from a treatment plant in Saskatchewan, Canada. Analytical conditions such as the ionization source, chromatographic column, and sample pretreatment were optimized and 553 brominated and 112 iodinated DBPs were detected. Predicted precursor ions and formulae exhibited variations in *m/z* values (170.884 – 497.0278), retention times (2.4-26.2 min) and in the number of incorporated halogen atoms (1-3). To better understand complex DBP mixtures and their toxicities, we compared source, clearwell (filtered), and finished (chlorinated) water that was sampled over the course of a year. Temporal trends and relationships were found for unregulated halogenated DBPs and NOM characterized by Orbitrap MS. Additionally, this chemical data was integrated with results from two in vitro toxicity assessments: a 72 h CHO-K1 cytotoxicity assay and an Nrf-2 oxidative stress assay. The toxicity of source water could not predict the toxicity of finished water, which suggests that disinfection alters the toxic pathway involved. With the integration of data on temporal DBP profiles, NOM precursors, and toxicity endpoints we hope to elucidate what NOM precursors are most important to remove prior to chlorination to reduce the occurrence of unregulated halogenated DBPs that present the greatest concern to human health.

349 Transformation and fate of neonicotinoid insecticides during drinking water treatment

K. Klarich, D. Cwiertny, D. Webb, G.H. LeFevre, University of Iowa / Civil and Environmental Engineering

Presentation Type: Oral Preferred Track: Environmental or Analytical Chemistry Session: Integrated tools for improving environmental fate and risk assessment for unregulated contaminants and their mixtures Abstract Title: Transformation and fate of neonicotinoid insecticides during drinking water treatment Authors: Kathryn L. Klarich, David M. Cwiertny, Gregory H. LeFevre, Danielle Webb Abstract: Despite their widespread detection in surface waters, relatively little is known about neonicotinoid fate in the environment. Neonicotinoids have been identified in water resources used for drinking water, but their potential for treatment and removal via the physical, chemical, and biological processes used in conventional drinking water treatment systems is unknown. This work measures the transformation and removal of common neonicotinoids via the physical and chemical processes used in drinking water treatment. We demonstrate that many of these processes are unlikely to remove neonicotinoids and may alter their structures so as to remove their specificity to invertebrates. This transformation could expose non-target organisms, including humans, to unanticipated risks arising from their bioactive transformation products. Through laboratory batch experiments and monitoring at two drinking water treatment plants, we assessed the fate and transformation of neonicotinoids through conventional treatment processes. In ongoing work, we are identifying transformation products and assessing the effectiveness of granular activated carbon (GAC) as a potential treatment technology for neonicotinoids. The results of this work will be used to preliminarily evaluate potential risks posed to humans and other non-target organisms by neonicotinoids in drinking water resources.

350 Environmental Photochemistry of Dienogest

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In an ongoing effort to study the environmental fate of potent, endocrine active steroid hormones, we report on the photochemical fate of dienogest, a dienone progestin used as a next-generation oral contraceptive. Our previous studies have shown that potent trienone and dienone steroids undergo a photohydration (in light)-thermal dehydration (in dark) cycling that ultimately results in increased environmental persistence by regenerating the parent steroid from phototransformation products in the absence of light. In this study, we used advanced analytical chemistry techniques (e.g., high resolution mass spectrometry and both 1D and 2D nuclear magnetic resonance spectroscopy) to monitor dienogest photochemical decay and also elucidate the structure and monitor the stability of the transformation products formed. Dienogest undergoes relatively rapid direct photolysis ($t_{1/2}$ ~ 1-11 min), resulting in the formation of complex mixtures of products across the pH range examined (2-7). Some of the products identified include three photohydration products, which account for ~ 80% of the converted mass at pH 7 and were found to revert back to parent dienogest in the dark after photolysis. We also identified two known estrogenic compounds that formed via A-ring aromatization of dienogest, demonstrating photochemical conversion of a progestin to an estrogen that would impart residual estrogen receptor activity. In addition, we discovered an unusual phenolic photoproduct produced by rearrangement of the steroidal core structure to yield a novel tetracyclic ring system. Overall, these results imply that dienogest will undergo complete and facile photolytic transformation in sunlit surface water, yet exhibit increased environmental persistence through a diurnal photohydration-thermal dehydration cycling while also producing transformation products with different biological endpoints (i.e., progestin to estrogen conversion). Collectively these outcomes reveal a dynamic fate profile for dienogest that challenges our current regulatory, risk assessment, and monitoring approaches.

Birds Under Stress – Integrative Studies for Understanding Effects of Environmental Pollution in the Wild – Part 1

351 Carolina Wren Exposure to Mercury: Integrating Stressors and Habitat Conditions

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Legacy mercury (Hg) from 1929-1950 in the South River (SR), Virginia, has been extensively studied over the last four decades. The South River Science Team (SRST), a multi-stakeholder collaborative program, was established in 2001 to investigate the potential impacts to the aquatic and riparian systems at the site. Based on the SRST studies, elevated Hg concentrations observed in the blood from the vermivorous song bird, Carolina wren (*Thryothorus ludovicianus*), along the riparian corridor have been attributed to their diet consisting of spiders that prey on emergent aquatic insects from the SR. As a result, the wrens are potentially at risk due to Hg and also form an important link in the Hg trophic transfer from aquatic to adjacent terrestrial systems. To evaluate the potential risk toward wren populations at a watershed level, risk factors other than Hg may be important. In this study, a modelling framework was applied based on the Bayesian Network-Relative Risk Models (BN-RRMs) that integrated different stressor types (Hg and winter air temperature) and environmental factors (habitat type and nest predation). Five contiguous risk regions were defined, including an upstream reference region, along ~40 miles of the river system. The model was run using historical data on wren blood Hg and other factors to establish baseline conditions; recently collected post-remediation data will be evaluated using the same model. The approach, calculations, and the findings will be presented. Results for historical data indicate that: 1) environmental conditions (habitat type and nest predation) are important considerations in evaluating the overall risk, although Hg is the primary chemical stressor, 2) relative risks vary among the regions due to their differences in Hg exposure and other factors including habitat quality/availability, 3) remediation to address Hg may reduce the overall risk by varying degrees depending on the non-Hg factors. Hence, the BN-RRM for the wren is a demonstrably promising tool to: 1) communicate a holistic risk evaluation approach, 2) integrate stakeholder priorities in decision making, and 3) to support remedial decision making and adaptive management at complex contaminated sites.

352 Explaining variation in songbird blood mercury using diet, foraging behavior, migratory behavior, and phylogeny

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Increasingly, contaminants such as lead, mercury, and PCBs, make their way into water, soils, and the atmosphere where they impact animals and pose challenges for wildlife conservation. Environmental contaminants affect species differently, and furthermore, species bioaccumulate contaminants to varying degrees which may put certain species at greater conservation concern. Here we investigate interspecific variation in mercury bioaccumulation in songbirds. Mercury has been studied in songbirds previously; however interspecific variation in mercury levels is not fully understood. Although mercury is known to biomagnify up food chains, trophic level alone does not always explain the variation observed in songbirds. Our study investigated the effects of trophic level, foraging behavior, phylogeny, and migratory behavior on bioaccumulation of mercury in songbird species. To our knowledge, these factors have not been studied all together on songbird mercury bioaccumulation in the same system previously. We found that foraging behavior affects mercury levels with ground foraging species accumulating the greatest amount of mercury, perhaps as more predatory insects are found on the ground compared to other foraging guilds. Additionally, long distance migrants had the greatest mercury levels which may be due to many migrants being year-round carnivores and accumulating more mercury over the course of the year. We found carnivorous species had the highest mercury as is expected due to biomagnification. Additionally, phylogeny also relates to mercury bioaccumulation with closely-related species exhibiting similar mercury levels. We suggest that long distance migratory species such as Common Yellowthroat and Wilson's warbler, and carnivorous species feeding at high trophic levels may bioaccumulate mercury to dangerous levels.

353 Environmental correlates and effects of organochlorine pollutants on the physiological condition and carotenoid-based coloration of an avian predator

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Contaminants such as PCBs and DDTs have caused population declines in avian predators. Banned in the 70-80s, their use persists in some countries. In South Africa, PCBs were still used in electric transformers in 2010. DDT was also intensively (illegally) sprayed in agricultural areas until 1985 and is currently legally used in the northeast of the country for malaria control. Residues from former and current use of PCBs and DDT therefore likely persist in the environment. Understanding patterns of OC contamination can be crucial for the conservation of wild animals such as African raptors. The Black Harrier *Circus maurus* is an endangered raptor endemic to southern Africa. This bird breeds in indigenous vegetation of southwestern South Africa, where agriculture and urbanization have rapidly developed since the 50s. We collected blood samples from wild nestlings (n=90) and adults (n=23) in 2012-2014. PCBs and DDT were detected in 79% and 84% of sampled individuals, respectively. Nestlings had higher ΣPCB and p,p'-DDT levels than adults, which in contrast presented higher levels of p,p'-DDE than nestlings. ΣPCB Levels increased with an index of "electric transformer density", which combined the number and power of electric transformers around active nests. Levels of p,p'-DDE increased with the % of wetlands within the breeding territory, suggesting bioaccumulation in sediments, and with the % of bird biomass in diet, confirming intra-specifically an association between diet and DDT contamination. Several indicators of physiological condition were affected in contaminated individuals: white blood cell count increased with higher p,p'-DDT levels and the heterophyl:lympocyte ratio increased with higher ΣPCB levels. In nestlings, ΣDDT levels reduced the amount of circulating carotenoids and the orangeness of colored integument developed by nestlings. Our results suggest that OCs can increase physiological stress and affect immunity, and may disrupt carotenoid-based signalling in exposed nestlings interfering with communication processes. OCs are therefore still a current cause of concern for threatened Black Harriers and other sympatric predators.

354 A spatially explicit model for estimating risks of pesticide exposure to bird populations

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Pesticides are used widely in US agriculture and may cause effects to non-target organisms, including birds. Some pesticide classes (e.g., acetylcholinesterase inhibitors) are known or suspected to cause direct mortality to birds, while others (e.g., synthetic pyrethroids, neonicotinoids) may cause sublethal effects, such as impacts to reproduction. Recently, the United States Environmental Protection Agency has worked with other federal agencies, including the US Fish and Wildlife Service and National Marine Fisheries Service, to revise and strengthen methods for conducting pesticide risk assessments under section 7 of the U.S. Endangered Species Act (ESA). We developed an integrated modeling approach for spatially explicit population level risk assessment for birds in agroecosystems that could be used to assess risks of pesticides to avian species, including those listed as threatened or endangered under ESA. The integrated modeling approach includes the following three existing USEPA models: Terrestrial Investigation Model (TIM), Markov Chain Nest Productivity Model (MCnest), and HexSim model. The integrated model is parameterized using data currently required by the Federal Insecticide Rodenticide Act for registration of pesticides, together with species life history data available in the scientific literature. We demonstrate the model by simulating potential effects on the federally threatened California Gnatcatcher (*Polioptila californica*) in the US portion of the species range using malathion (an organophosphate), and λ-cyhalothrin (a pyrethroid), applied to wheat under varying spatially explicit usage consistent with the labeling for the two insecticides. Within the range of input parameters used, the models predict declines in gnatcatcher abundance and changes in the distribution of the species following applications of each pesticide, although extinction of the species is not predicted (within 100 years). The integrated TIM/MCnest/HexSim model will allow risk assessors to evaluate spatial and temporal dynamics that are essential to understanding population persistence in complex spatial landscapes with multiple stressors.

355 Current-use pesticides and adult aquatic insect prey flux to terrestrial food webs in Prairie Potholes Wetlands

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Current-use pesticides can impact ecosystems by reducing the quantity and quality of non-target prey organisms available to aquatic and terrestrial consumers. Because freshwaters can act as receiving basins for contaminants and are well connected with the surrounding landscape through movements of predators, prey, energy and nutrients, they may be hotspots of non-target effects of pesticides on food webs. The Prairie Pothole Region of North America encompass a system of wetlands that are both crucial breeding and feeding grounds for migrating birds, and are embedded in a landscape predominantly used for cropland agriculture. Adult aquatic insects emerging from these wetlands are important prey for ducklings, aerial insectivorous birds and aquatic predators such as fish, and can act as vectors of contaminant transfer to multiple food webs. We surveyed wadeable wetlands in cropland and grassland landscapes across a salinity/hydrology gradient (N = 14 in 2015, N = 15 in 2016) to test the effects of land use and wetland type on pesticide accumulation and flux in adult aquatic insects. We also tested the relationship between pesticide concentration in insect tissue and aquatic insect emergence (mg insect/m^2/day) during the bird breeding season. Preliminary results suggest that 1) current-use pesticides including atrazine, bifenthrin and imidacloprid accumulate in adult aquatic insect tissues, 2) insects emerging from cropland wetlands have more pesticides in their tissues than insects from grassland wetlands, 3) aquatic insects flying over grassland wetlands contain more pesticides in their tissues than those captured emerging from them and 4) summed insecticide concentrations in adult insect tissues are negatively correlated with emergence production during the bird breeding season. Current-use pesticides likely contribute to the effects of agricultural land use on linked aquatic-terrestrial food webs. Landscape models of aquatic insect emergence and contaminant flux across the Prairie Pothole Region could aid in management of waterfowl and other avian consumers.

356 Findings of a study examining kinetics and toxicity of neonicotinoid coated seeds to seed-eating birds

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Globally, neonicotinoids are the most widely used insecticide. They act as agonists of nicotinic acetylcholine receptors, which can result in paralysis and eventual death in exposed insects. Toxicity for non-target wildlife appears to be lower than for invertebrates. Nevertheless, neonicotinoid studies in birds have reported genotoxic, cytotoxic, immunological, behavioral and reproductive effects. Remarkably little is known about neonicotinoid kinetics (absorption, distribution, metabolism and excretion) in birds, which can dictate the time course for toxicity. Using Japanese quail (*Coturnix japonica*) as a model avian species, we investigated kinetics and toxicity of a pesticide formulation coated on wheat seeds that principally included the neonicotinoid imidacloprid ($< 10^{-3}$ of the avian LD50/seed) and low levels of 3 fungicides ($\leq 10^{-7}$ of the avian LD50/seed). Using radiographic technology to view radiopaque-coated seeds in the gastrointestinal tract, rapid removal of coatings and digestion of seeds was observed (< 2h). We estimated Japanese quail could ingest a maximum of 30 seeds (i.e., < 10% of LD50 for imidacloprid) in a single foraging bout. Quail were orally dosed with wheat seeds for 1 day or daily for 10 d as follows: (i) sham-dosed (30 untreated seeds), (ii) low dose (10 treated and 20 untreated seeds), and (iii) high dose (30 treated seeds). Immediately prior to euthanasia, the escape response and take-off performance of birds was assessed. Birds were euthanized 1, 2, 4 and 24 h after a single dose, and 1, 4 and 24 h after 10 consecutive days of dosing. LCMS/MS analysis revealed imidacloprid concentrations 1 h post treatment were similar in plasma and liver. Clearance from plasma was rapid (single dose, elimination half-lives were estimated at 1-7 h (low) and 1-5 h (high); 10 doses, the equivalent ranges for low and high doses were 5-9 h and 1-5 h). Imidacloprid in plasma was only detectable in 2 of 16 birds 24 h post-exposure. After a single dose, birds in the high dose group were more likely to perform poorly in escape response tests than the controls and low dose groups (P< 0.01). Following 10 d repeated exposure (realistic, but worst case scenario), we found no evidence of effects on plasma corticosterone levels or oxidative DNA damage (8-OHdG) in liver, blood or brain. These data are among the first to investigate dose-response and kinetics of imidacloprid any bird, making them highly pertinent to current needs of ecological risk assessment.

357 Uptake and metabolism of imidacloprid in Japanese quail following ingestion of treated wheat seeds

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Advancements in seed treatment technologies and formulations have led to nearly all major crop seeds being directly coated with pesticides. Seed coatings have reduced the need for surface applications of pesticides, decreasing aerial exposure to farmers and wildlife. However, there is potential for high-level exposure of foraging birds via the direct ingestion of treated seeds. Therefore, it is essential to understand the fate and effects of seed treatment pesticides and their metabolites in non-target organisms. In this laboratory exposure study, Japanese quail (*Coturnix japonica*) were used as a model avian species. Quail were orally dosed with wheat seeds for 1 day or 10 consecutive days in control, low dose (10 treated seeds), and high dose (30 treated seeds) groups. Treated wheat seeds contained a neonicotinoid insecticide (11.16% imidacloprid) and three fungicides (0.60% metalaxyl; 0.45% tebuconazole; 0.36% fludioxonil). Birds were euthanized 1 h post-gavage, and plasma and tissue (liver, brain, muscle, and kidney) samples were harvested. Tissue samples were homogenized and extracted via pressurized liquid extraction. To remove protein and lipid interferences, precipitation and pass-through solid phase extraction (SPE) clean-up steps were performed. Plasma samples were extracted and cleaned-up via protein precipitation and pass-through SPE. Samples were analyzed by LC/MS/MS for all parent pesticides and three imidacloprid metabolites: 5-OH-imidacloprid, imidacloprid-olefin, and imidacloprid-urea. Imidacloprid, 5-OH-imidacloprid, and imidacloprid-olefin were detected in the plasma and livers of all exposed Japanese quail, whereas imidacloprid-urea was not detected in any sample. Concentrations of imidacloprid, 5-OH-imidacloprid, and imidacloprid-olefin were significantly greater in the high dose group compared to the low dose group, but no significant differences were observed between 1 and 10 day dosing regimens. Moreover, concentrations of the metabolites were an order of magnitude greater than the parent compound. Imidacloprid has exhibited moderate to high acute oral toxicity in birds, but much less is known about the effects of imidacloprid metabolites; which may have a greater potency. Understanding the effects and fate of seed treatment pesticides and their transformation products in this model species will enhance interpretation of detected pesticide residues from field studies.

358 Tracking the effects of a neonicotinoid insecticide on migratory songbirds

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Recent decades have seen a dramatic increase in the application of neonicotinoid insecticides, which are now the most widely used class of insecticides in the world. Birds that travel long distances between their wintering and breeding grounds may be particularly susceptible to the neurotoxic effects of neonicotinoids. However, the influence of neonicotinoids on the ability of birds to successfully migrate is poorly understood. We assessed the direct effects of imidacloprid (IMI; a neonicotinoid commonly used in seed treatments) on the migratory behavior of seed-eating passerines during their spring migration. In 2016, we used captive orientation funnel trials to measure migratory orientation and activity of White-crowned Sparrows (*Zonotrichia leucophrys*) caught at stopover sites, and found that birds exposed to environmentally relevant concentrations of IMI reduced food consumption, experienced significant mass loss, and stopped orienting correctly in behavioural trials, whereas control birds maintained body mass and a seasonally appropriate northward orientation. In 2017 we conducted a field study in free-living White-crowned Sparrows to corroborate results from captive trials at an ecologically relevant scale. Birds were caught at a stopover sight in southern Ontario and exposed to control, low, or high doses of IMI (equivalent to 1 or 4 treated canola seeds). Following exposure birds were tagged with uniquely coded transmitter tags and released into an array of automated telemetry towers (Motus Wildlife Tracking System) to track their movements at a landscape scale. Using manual and automated telemetry data, we will be able to assess stopover duration, speed of travel between points, and direction of migratory movements.

Stormwater and Wastewater Pollution – Toxics, Ecological Sensitivity and Sustainable Solutions for a Healthy Environment

359 Fate of estrone under anammox conditions: By-product formation and aquatic toxicity to fathead minnow larvae

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The presence of estrogenic chemicals in wastewater influent presents a challenge to conventional municipal wastewater treatment processes, as they were not designed for the removal of these compounds. In recent years anaerobic ammonium oxidation (anammox), a relatively new treatment technology, has captured worldwide interest as a sustainable, low-energy method for total nitrogen removal from wastewater. This research investigated the aquatic fate of estrone (E1), a common estrogenic contaminant present in wastewater, in a sequencing batch reactor operating under anammox conditions. Despite the anaerobic nature of the anammox process, excellent E1 removal was achieved (95% mean E1 loss) without the anticipated estradiol (E2) formation. Reactor effluent was concentrated, derivatized, and analyzed via gas chromatography coupled to mass spectrometry (GC-MS) for the identification of by-products and characterization of their formation/transformation kinetics. In addition, fathead minnow larvae were exposed to reactor effluent in a 30-day aquatic toxicity assay, to evaluate the toxicity of identified and unidentified products formed during E1 degradation in anammox conditions. This research should enable the application of a new low energy treatment technology for total nitrogen removal while simultaneously evaluating the fate of co-occurring estrogenic wastewater pollutants.

360 Effectiveness of a Constructed Wetland in Removing Emerging Contaminants from Municipal Wastewater

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Contamination of water bodies by pharmaceutical compounds and other emerging contaminants is now widely considered to be ubiquitous. The vast majority of these compounds enter the environment through treated wastewater discharges from septic and sewer systems. Unfortunately, traditional wastewater treatment processes are not designed to remove these emerging contaminants, and implementing tertiary treatment methods specifically for their removal is often prohibitively expensive. Wetlands have been shown to effectively remove nutrients and other contaminants from wastewater, along with providing various co-benefits. This project, which is part of the Sewanee Wetlands Project, is a pilot study that investigated the potential for using a constructed wetland system to remove emerging contaminants from lagoon-treated wastewater in the small rural setting of Sewanee, Tennessee. Wastewater samples obtained from Sewanee's facultative final wastewater treatment lagoon and four locations within the constructed wetland were analyzed by LC-MS/MS for 19 model emerging contaminants. Preliminary results suggest that the constructed wetland may provide effective removal of pharmaceuticals and other emerging contaminants. However, there may be seasonal variability associated with the removal efficiencies of contaminants. Higher mean removal efficiencies (> 48%) of the analytes were observed in warmer weather, which may be due to light availability for photolysis, microbial degradation, and vegetative factors. Additionally, the hydrophobicity of the compounds detected in the wetlands may have influenced their individual removal efficiencies, likely through influencing adsorption to sediment. A statistically significant relationship was observed between compounds' log K_{ow} values and percent removal efficiencies. These preliminary results show that constructive wetlands may be a cost-effective method to remove certain classes of pharmaceutical contaminants from wastewater streams.

361 Assessing Load Reduction and Biological Recovery After Wastewater Treatment Upgrades in an Effluent-Dominated Aquatic Ecosystem

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Many urban aquatic systems are becoming effluent dominated, resulting in the presence of contaminants of emerging concern and subsequent adverse effects on aquatic wildlife. Despite these dramatic alterations, effluent dominated urban systems support many ecosystem services and are used by the nearby human population for recreation. The Metropolitan

Water Reclamation District of Greater Chicago is upgrading two billion liter/day wastewater treatment plants to disinfection (UV; chlorination/de-chlorination). The receiving aquatic ecosystem adjacent to these two wastewater treatment plants has been the focus of intense biological and chemical study for the past seven years and provides a unique opportunity to assess two divergent treatment technologies (UV disinfection vs. chlorination/de-chlorination) and to examine how adverse biological effects in exposed fish may be mitigated through effluent disinfection. We exposed male fathead minnows in on-site flow-through exposure systems four times prior to the treatment upgrades and four times since to examine these questions. In addition, we conducted extensive analytical chemistry on effluent samples and employed in-vitro assays to examine overall biological activity of effluents prior and following disinfection treatment. UV disinfection resulted in an improved maturity of male fathead minnows when compared to fish exposed to non-disinfected effluent or a control treatment ($P< 0.05$, ANOVA). Disinfection by chlorination/dechlorinating reduced testis maturity and changes liver hepatocyte appearance (both $P< 0.05$, ANOVA). These data suggest that biological activity of effluent is altered by both disinfection treatments but with more advantageous outcome using UV disinfection.

362 Juvenile coho salmon as a surrogate for adult spawners in investigation of acute lethal response to urban stormwater runoff

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Urban stormwater runoff is a leading source of nonpoint pollution due to its ability to quickly mobilize a diverse mixture of contaminants into aquatic environments. Direct exposures to highway runoff are known to cause acute mortality in both adult and juvenile coho salmon (*Oncorhynchus kisutch*). Coho exposed to urban runoff display a common suite of symptoms including surfacing, gaping, and a loss of equilibrium. Previous research profiling adult coho blood gas and chemistry during urban runoff exposure indicated disturbances in acid-base and ion regulation. This study investigated the utility of juvenile coho salmon as a surrogate for adult spawners to understand the acute lethal response observed during urban runoff exposure. Juvenile coho were exposed to collected urban runoff or clean control water. Runoff fish were sampled at three behavioral stages (asymptomatic, surfacing, and loss of equilibrium) with time matched controls. Surface behaviors were semi-quantitatively characterized and compared with behaviors observed in adult salmon. To investigate the physiological response, fish were sampled for arterial blood and tissues. Arterial whole blood, red blood cell lysate, and plasma were analyzed for blood gases and chemistry using an iSTAT point-of-care analyzer. Gill and kidneys were collected for Na/K and H+ ATPase activities. Juvenile behaviors induced by runoff exposures were comparable to those observed in adults and progressed through a distinct pattern starting with discrete surfacing events and eventually leading to a loss of buoyancy. In addition, the blood profile of runoff-exposed fish indicated acid-base and ion regulation disturbances previously characterized in adults. Together, the behavior and physiological response supports the use of juveniles as a surrogate model to adult spawners.

363 Impacts of urban stormwater runoff on development of nearshore marine fish embryos

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Pacific herring (*Clupea pallasii*) are a keystone species that spawn adhesive eggs on intertidal and shallow subtidal substrates. This nearshore spawning places sensitive life history stages (embryos and larvae) in close proximity to land-based non-point source pollution such as urban stormwater runoff. Untreated urban runoff is chemically complex and highly toxic to aquatic life, including freshwater fish and invertebrates. However, very little is known about the impacts of urban runoff on nearshore marine fish. To examine the impacts of stormwater runoff on forage fish embryonic development, we exposed herring embryos to 0, 12, 25, or 50% stormwater runoff beginning just prior to the onset of a visible heartbeat (5 dpf) through hatching (11 dpf). Preliminary results indicate that stormwater exposures caused significant reductions in larval length and greater egg yolk area, consistent with a failure to mobilize embryonic energy stores (yolk). In addition, herring exposed to stormwater runoff exhibited cardiac injury including both functional (e.g., bradycardia, contractility) and morphological (e.g., decreased ventricle area) heart defects. The observed effects are consistent, in part, with the known cardiotoxicity of polycyclic aromatic hydrocarbons to fish embryos. In summary, stormwater runoff causes altered embryonic growth and cardiac injury in Pacific herring embryos. These effects could result in delayed adverse outcomes such as reduced cardiorespiratory fitness and subsequent mortality.

364 Suspect and non-target screening of organic contaminants in highway runoff and stormwater treatment systems

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Urban runoff transports chemical contaminants, toxicants, and other bioactive chemicals, thus contributing to poor water quality in receiving waters. To protect ecosystem and human health, identification of contaminants and toxicants in urban runoff is a necessary but challenging analytical task. Further, as low-impact, green infrastructure is often used to treat stormwater runoff in the urban environment, thorough evaluations of the performance and effectiveness of such systems are critical. Here, we present the use of high resolution quadrupole time of flight mass spectrometry to characterize the occurrence and fate of trace organic contaminants, including novel compounds, in stormwater and highway runoff and to evaluate performance of bioretention systems for stormwater treatment. To develop a non-target "chemical fingerprint" for organic contaminants in stormwater runoff, we evaluated the performance of stormwater treatment systems, including a compost-amended bioswale and a bioretention column, and analyzed paired sets of highway runoff samples pre- and post-treatment. After data reduction and prioritization, we tentatively detected >20 compounds in the highway runoff across a number of chemical classes, including pesticides, corrosion inhibitors, industrial chemicals, and natural products. Of those non target features common to multiple batches of untreated stormwater runoff, 76% and 93% were removed in the bioswale and bioretention column, respectively. While removal of specific compounds differed between the two systems (e.g., 100% removal of the corrosion inhibitor tolytriazole in the bioretention column versus ~30% removal in the bioswale), we observed similar overall trends in removal across compound hydrophobicity (i.e., $\log K_{ow}$, predicted by retention time of non-target features) for these short hydraulic retention time, sequestration based treatment options.

365 Identifying the Sources of Toxicity to Urban Road Runoff

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Urban stormwater runoff contains a complex mixture of contaminants that is toxic to aquatic animals, including acute mortality in adult coho salmon spawners. In an embryo-larval fish model (zebrafish; *Danio rerio*), urban stormwater runoff was previously shown to be developmentally toxic, particularly to the cardiovascular system. Sub-lethal effects caused by exposure include developmental delay and microphthalmia, as well as cardiovascular abnormalities including improperly looped hearts and pericardial edema. The contaminant(s) causing these effects is currently unknown. One approach to identifying the relevant contaminants is to first isolate the compartment in which the contaminant is found. The majority of chemical contaminants in road runoff are derived from motor vehicles, including a number of particulate and liquid sources. We identified sources that are expected to contribute the most contaminants to road runoff. The particles included tire dust, brake dust, and vehicle exhaust. The liquids included windshield washer fluid, antifreeze, motor oil, transmission fluid, brake fluid, and power steering fluid. Samples of each source were tested for dose-dependent acute lethal and sublethal toxicity using zebrafish embryos. Embryos were exposed to dilutions of each source for 48 h beginning at the blastula period (2-5 hours post-fertilization). At 48 h, surviving embryos were removed from their chorion and imaged for later analysis from digital stills and video. Survival and sublethal metrics including embryo length, eye area, and heart-related metrics were analyzed to determine dose-response metrics for each source. Brake fluid and transmission fluid are the most acutely lethal of the fluids (LC50 = 0.4% and 0.5% v/v, respectively), followed by antifreeze and used motor oil (1.8% v/v), and windshield washer fluid (5.3%). The most sensitive sub-lethal endpoint was the cardiovascular system, where the most acutely toxic fluid was used motor oil (heart effects at 0.04% v/v; 2% of the LC50), whereas the other fluids were sub-lethally toxic much closer to or above their LC50. Results from the dusts will be presented as well as an initial estimation of the relative contribution of each component to the mixture of urban stormwater runoff.

366 Effects of selective serotonin reuptake inhibitors sertraline and citalopram on hybrid striped bass predatory behavior and brain/intestine chemistry

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Antidepressants are commonly prescribed drugs in the United States. Selective serotonin reuptake inhibitors (SSRIs) are one of the most prescribed antidepressant classes. Waste water treatment facilities are often ill-equipped to handle the removal of pharmaceuticals from incoming waters, leading to the discharge of SSRIs into the environment. Fish populations around discharge pipes could be experiencing exposures to sub-lethal concentrations (low ppb to high ppm) of SSRIs which could affect their ecological fitness in the environment. The goal of this research is to determine whether SSRIs cause sublethal effects on fish populations through a change in feeding behavior, further quantified by analyzing brain chemistry and changes in gene expression in the intestine. Past research of SSRIs on hybrid striped bass (HSB) showed an increase in time to capture prey explained by a decrease in serotonin concentrations in the brain. We hypothesize a decrease in feeding behavior, a decrease in serotonin levels, and a change in gene expression after exposure to sertraline and citalopram. HSB were exposed to citalopram (50-150 µg/l) and sertraline (4-100 µg/l) followed by feeding periods every three days (3, 6, 9, and 12 days) to determine effects on behavior. Blood, brain, and intestine samples collected from euthanized fish every three days were analyzed for concentrations of citalopram, sertraline, and serotonin. Both sertraline and citalopram showed a change in predatory behavior, with sertraline having a more dramatic effect than citalopram. Our results are suggesting SSRIs may cause an up-regulation of both the serotonin reuptake transporter and cholecystokinin. From an ecological standpoint, an increased feeding time could make exposed bass populations less ecologically fit compared to other fish populations not as affected by the antidepressants.

Unique Laboratory and Field-Based Methods to Address Complex Environmental Issues

367 Estrogenic activity, estrogens, and calcium in runoff post-layer litter application from rainfall simulated events

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Estrogens in runoff from fields fertilized with animal wastes have been implicated as the endocrine disruptors of fish in recipient surface waters. The goal of this study was to measure estrogenic activity in runoff post-application of the animal waste with the greatest potential for estrogenic activity - fresh layer hen litter from caged chickens. Litter was applied and tilled into six 0.0045 ha plots. Six alternating plots were tilled (controls), but without addition of hen litter. The next day, rainfall simulations were executed (RFS) and runoff was collected from treated plots at 10, 15, 20 and 30 min, and composites were taken from the total runoff event for both control and treated plots. Rain events were repeated 1 and 3 wks after the initial event. Samples were analyzed for estrogenic activity (E-Screen), estrogens, calcium (Ca), coliforms, and E.coli. Previous work indicated a possible correlation between Ca and estradiol equivalents (E2Eq) in runoff. The maximum E2Eq never exceeded 3 ng/L and decreased to ≤ 0.1 ng/L by week 3. In contrast, mean soluble Ca increased over events from 18 to 46 mg/L in treated plots (controls 14 to 15 mg/L). Because peak E2Eq and Ca were not concomitant, Ca would not be a useful surrogate for indicating the presence of E2Eq in runoff post-layer litter application. Soil moisture influenced coliform number. Drier plots had lower coliform numbers, though variability among plots was extreme (37 fold for controls and 25 fold for treated). Similarly, E.coli numbers were related to soil moisture, with the highest counts in week 3 runoff in 4 of 6 plots. While ß-E2 was present in litter, no β-E2 was detected in runoff. Maximum 17α-E2 and estrone concentration in runoff was ~ 3 and 14 ng/L respectively, lower than concentrations documented to induce vitellogenin in fathead minnows. Our results indicate runoff post-application of *composted* layer litter should not cause estrogenic endocrine disruption, as concentrations post-application of "fresh" litter were lower than necessary to elicit disruption.

368 Application of novel in-situ denitrification measurement in land-based industry wastewater treatment systems to inform regulation development

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The vegetable processing and dairy industries are vital to Wisconsin's economy, but their operations create large volumes of wastewater. In 2014, Wisconsin's vegetable processing industry generated an estimated 2.4 to 5.5 billion gallons of wastewater from processing snap beans, sweet corn, and green peas and the cheese industry approximately 5.8 billion gallons to produce 2.9 billion pounds of cheese. Land treatment has been used for decades to treat and dispose of wastewater generated by processed vegetable and cheese production facilities. Although many facilities utilize highly engineered systems, land application is still commonplace throughout the country. These soil-based systems are relatively simple and easier to manage at lower cost than highly engineered approaches. Through careful characterization and monitoring of wastewater and application site parameters, long-term wastewater land application can be performed in an environmentally sustainable manner. Wisconsin statute NR214 provides primary guidance on land treatment systems stating, "Total lbs of nitrogen applied per acre per year shall be limited to the annual nitrogen needs of the cover crop, plus demonstrable nitrogen losses, such as from denitrification or ammonia volatilization…" However, 'demonstrable nitrogen losses' is not quantified and industry and regulators are at odds over the amount of denitrification occurring. Our industry and regulator jointly-funded 3-year research project is in the process of developing full nitrogen budgets for the land treatment systems used by vegetable processors (spray fields) and cheesemakers (ridge and furrow). In addition to measuring wastewater N inputs, N plant uptake, soil storage and leaching, a novel application of an acetylene inhibition N_2O measurement method is being used to quantify denitrification. Networks of four soil gas collection chambers designed according to the USDA-Agricultural Research Service's GRACEnet protocol have been placed within treatment fields. A photoacoustic gas analyzer attached directly to the sampling chambers measures nitrogen gas fluxes in-situ over seven-day periods associated with single load/rest cycles in the spring, summer, fall and winter. This data is being collected at six facilities in different regions and soil types throughout Wisconsin. Our results will be used to refine regulatory guidance and permitting and will have direct impact on industry operations state-wide.

369 Photoperiod, exposure duration, and latent mortality: Photo-induced toxicity effects in aquatic organisms

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Polycyclic aromatic hydrocarbons (PAHs) are a class of organic contaminants composed of two or more fused carbon rings and are a major constituent of crude oil. Exposure to ultraviolet radiation (UV) can exponentially increase the toxicity of photodynamic PAHs to biota, leading to adverse outcomes well below the threshold of other mechanisms of toxicity. This phenomenon is known as photo-induced toxicity and is well documented in a wide range of aquatic organisms. Consequently, laboratory tests investigating effects of PAH on aquatic biota which fail to account for potentiation by UV radiation may significantly underestimate toxicity. The intensity of UV light exposure to biota is highly variable within aquatic ecosystems due to a number of factors intrinsic to the water column (e.g. dissolved organic matter content, photo-bleaching, phytoplankton, and detritus), as well as extrinsic factors (e.g. cloud cover, time of day, seasonal variations). Tissue repair mechanisms may also be sufficient to counteract some of the effects of photo-induced toxicity during periods of relief from UV exposure. Here, we report the results of experiments in which larval fish (red drum *(Sciaenops ocellatus)*)and zooplankton (*Daphnia magna*) were exposed to either a single PAH (fluoranthene) or a complex PAH mixture prepared from weathered crude oil with varying PAH and UV exposure scenarios. In red drum, all tests were conducted as a single pulse exposure and in daphnia, tests were conducted as static renewals. Toxicity (LC_{50}) was UV and PAH dependent in both species. In red drum tests, shorter PAH pre-exposure times (before UV light was added) resulted in LC_{50}s that were considerably lower (sub-ppb) than LC_{50}s associated with longer pre-exposure periods. This is likely due to lag time in the initiation of physiological metabolism/clearance mechanisms in the organism and loss of PAH from the test chamber. A similar pattern was observed in red drum photoperiod testing. In daphnia, LC_{50} decreased with increasing photoperiod. Interestingly, significant latent mortality was observed in daphnia for several days following the conclusion of the UV and PAH exposures. Taken together, these data suggest that even short-term transient exposure to low concentrations of PAHs common during a spill event results in acute toxicity in aquatic organisms and those effects may be manifested outside of standard bioassay testing durations.

370 Applying Diffusive Gradients in Thin Films (DGT) to Study Mercury Bioavailability in a Stream System on the Southeastern Coastal Plain, USA

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Waste basins associated with processing facilities at the US Department of Energy, Savannah River Site (SRS) near Aiken SC received liquid effluents that contained Hg, which seeped through the soil, entered the groundwater and outcropped in the wetlands adjoining Fourmile Creek (FMC) on the SRS. We deployed diffusive gradients in thin films (DGT) for 5 days in three locations of FMC and two locations of a non-contaminated stream that served as a controlled site. We collected water and surface sediment at the first day of deployment. Biofilms were grown on flat plates suspended in the stream water using floating frames. Temperature, pH, ORP, DOC, sulfate, chloride were measured and total Hg concentrations were determined for all DGT, water, sediment, and biofilm samples. The FMC may have received Hg from the seepage basin because its total Hg concentrations in the water, sediment, and biofilms were statistically higher than the control stream. The Hg concentrations in DGT revealed the vertical profile of Hg concentrations in pore waters that is defined as labile Hg (LHg) in this study. Control locations presented relatively constant concentrations of LHg through the sampling interval (i.e., 3 cm above the water-sediment interface to 11 cm below it), but FMC profiles showed a sharp increase of LHg concentrations at the interface. Meanwhile, the DGT deployed at FMC2 had the highest LHg concentrations in the interface, and also the highest total Hg concentrations in the water and sediment. These results demonstrated the importance of this water-sediment interface as it reflected the accumulation and/or generation of LHg in the pore water gradient. Among all the water chemistries, sulfate concentration was the most important relative to LHg. A significant positive correlation between sulfate concentrations and LHg concentrations at the interface indicated the importance of sulfate-reducing bacteria and their contributions to Hg methylation in the sediment. We also found out a positive relationship between total Hg concentrations in the biofilm and LHg concentrations in the water-sediment interface. This contributes to our understanding of the geochemical influences on Hg bioaccumulation and the heterogeneous biomagnification among similar ecological systems. It also indicates the DGT accumulated Hg is a good indicator of the bioavailable Hg, and can be applied to monitor and assess remediation efforts for Hg pollution in aquatic environments in the future.

371 Using environmental DNA to understand population level effects of aquatic environmental contaminants

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Numerous lab based techniques have been used over the years to try and understand potential population level effects of environmental contaminants in the wild. These studies are essential as it's typically not cost effective to perform studies in the wild or perform a periodic census of wild populations using traditional mark-recapture studies. However, recent advances in biotechnology have allowed for increased detection sensitivity of genetic material in environmental samples. Both aquatic and terrestrial organisms constantly slough and excrete genetic material that makes its way into the surrounding environment. As a result, researchers have begun to use this genetic material in environmental samples, termed environmental DNA, to understand ecology, population densities, and biodiversity. Additionally, this technique has shown promise for detection of vulnerable low density populations of endangered species that may not be accurately detected using traditional population assessments. Finally, studies have shown that environmental DNA data correlates well with mark recapture studies. As a result, environmental DNA studies may provide an opportunity for environmental toxicologists to assess the population level effects of environmental contaminants using a cost effective, high sensitivity, and accurate technique that takes much less time than traditional means. But it is essential to ensure that assays are specific for the species of interest as closely related species may be present in the same samples. Once specificity has been confirmed, extra care must be taken to ensure samples are not cross contaminated in the field. Finally, samples must be extracted and analyzed using traditional or digital droplet quantitative PCR. This presentation will walk through the process

of developing and validating an environmental DNA assay as well as procedures for field collection of samples, extraction, and detection using an example for the endangered smalltooth sawfish. Environmental DNA represents a promising technique for environmental toxicologist to assess population level effects of environmental contaminants, a tool which could be integrated into ecological risk assessments and natural resource damage assessments.

372 Endocrine Challenges of a Midwest Upbringing: Exploring Early Life Agrichemical Exposures in the Lab and Field

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Waterways of the Midwestern United States are subject to seasonal agricultural runoff events that contain pesticides, pharmaceuticals, fertilizers and suspended sediments. Such mixtures present a risk to early life stages of fish inhabiting impacted watersheds. Given the difficulties of working with larval fish under natural field conditions, there is a limited understanding about the impacts of seasonal agricultural runoff on early life stages. Here we present findings from a combination of laboratory and in situ larval exposure studies to multiple spring runoff events in the Elkhorn River watershed from 2015 – 2016. In 2015, fathead minnow larvae were exposed to seasonal runoff under natural conditions in outdoor mesocosms at the Elkhorn River Research Station, then allowed to recover udner natural field conditions as agrichemical concentrations subsided. Along with changes in expression of androgen receptor and insulin-like growth factor 1, growth depression in larval mass and condition factor were common responses when minnow (Pimphales promelas) larvae were exposed to this complex and seasonally-dynamic agrichemical mixture. Interestingly, our findings also demonstrate compensation in both growth, and in the expression of some, but not all, endocrine responsive genes during the post-runoff season. Further investigation under laboratory conditons during the 2016 season revealed that the anti-androgenic and anti-estrogenic responses of minnow larvae were dependent on whether larvae were exposed to the aqueous or sediment matrices found within agricultural runoff. These finding demonstrate that the response of minnow larvae towards agricultrual runoff is much more nuanced than was originally anticipated, highlighting the importance of evaluating early life stressors under natural and laboratory conditions.

373 Effects of Deepwater Horizon crude oil on visual function in early life stage fishes

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The *Deepwater Horizon* oil spill released millions of barrels of oil into the Gulf of Mexico, coinciding with peak spawning periods of economically and ecologically important fish species, such as the mahi-mahi (*Coryphaena hippurus*), red drum (*Sciaenops ocellatus*), and sheepshead minnow (*Cyprinodon variegatus*). Downregulation of genes important in eye development and function, as well as morphological abnormalities have resulted from polycyclic aromatic hydrocarbons (PAHs) present in the oil, impacting fish vision. Mahi-mahi, red drum, and sheepshead minnow embryos were exposed to weathered crude oil and assessed for visual function using the flicker-fusion principle to monitor an optomotor response, with subsequent histological analysis taken of each larvae's retina. Oil-exposed larvae exhibited a reduced PAH-dependent optomotor response with a reduction in retinal layers that play an important role in visual function and image processing. The present study provides evidence that weathered crude oil affects the visual system in developing larval fish, and relates oil-induced histological effects to behavioral endpoints. This research was made possible by a grant from The Gulf of Mexico Research Initiative.

374 Can terrestrial songbirds be used to assess mercury levels in an adjacent aquatic environment?

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Birds have been suggested as bioindicator to detect environmental contaminants because of their ecology is well established and they are often high on the food chain and thus susceptible to biomagification. In aquatic ecosystems, the focus has been on bird species that feed directly on aquatic species, fish or emergent insects, but terrestrial birds living adjacent to contaminated sites have been shown to accumulate aquatic contaminants to equally high levels. Here we assess whether contaminant levels in terrestrial birds reflect the levels seen in aquatic organisms from an adjacent stream. Research was conducted on Fountain Creek, a stream on the Front Range of Colorado known to be variably contaminated with mercury. Mercury levels have been measured in fish and bryophytes and have been shown to increase with increasing elevation. Terrestrial songbird mercury levels are spatially correlated with mercury levels in bryophytes and fish. Selection of songbirds of particular ages, foraging guilds, and behaviors may improve the correlation. Using terrestrial birds to estimate aquatic contamination may be particularly useful in areas where fish or other aquatic species are not available.

Remediation/Restoration – Innovative Design and Monitoring Techniques

375 Integrated Restoration/Remediation of a Mercury Contaminated River

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Historically released mercury (Hg) accumulated in depositional areas along the banks of the South River, Virginia. Natural erosion of river bank soils with elevated Hg concentrations is a primary mechanism for legacy Hg loading to the aquatic ecosystem. As part of initial measures under the regulatory authority of the Resource Conservation and Recovery Act (RCRA) Corrective Action, river banks are being remediatedin an upstream-to-downstream sequence within an adaptive management framework. At previous SETAC meetings (2016) we reported on integrated restoration and remediation designs for the impacted South River banks. These designs strike a balance between the remedial objectives of RCRA and stakeholder objectives of maintaining riparian and near-bank aquatic habitat functions, improving access to the river for recreational activities, and minimizing disruption. Based on the outcome of collaborative design discussions between the involved parties, optimization and balancing of these objectives can be best achieved by focused soil removal actions in localized banks that contribute disproportionately to Hg loading. Less invasive enhanced vegetative stabilization designs have been developed for those banks that contribute relatively smaller Hg loading. Work on the first bank area was completed in February 2017. This presentation will provide an update on project progress to date and discuss lessons learned during implementation of the first bank area of the project.

376 Utilization of Drone-Based Imagery to Facilitate Stakeholder Understanding of Riverbank Conditions Related to Remediation in the South River, Virginia

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Mercury has accumulated in depositional areas along the banks and floodplain of the South River, Virginia, as a result of historical mercury releases from a textile manufacturing facility on the river. Natural erosion of river bank soils with elevated mercury concentrations is a primary mechanism for loading of legacy mercury to the aquatic and terrestrial ecosystems. As part of the Phase 1 Interim Remedial Measures (IRMs) under the Resource Conservation and Recovery Act (RCRA) Corrective Action, river banks in the first two river miles of the impacted area along the South River are currently being assessed and remediated in an adaptive management framework. The remedial strategy for a broad area such as a river system, like the South River, requires proactive communication with stakeholders in order to be successful. All phases of the remedial strategy, from conceptual design to construction, have included the involvement of stakeholders, such as state wildlife agencies and local municipalities. An interactive web-based map has been designed to display images of river bank conditions across large portions of South River in a user-friendly interface to facilitate stakeholder involvement and understanding. This convenient desktop tool allows users to effectively inspect riverbank conditions during all phases of the program. The high resolution images included in the web-based map are captured by the use of a drone; the images are precise enough to evaluate river bank characteristics in the context of the remedial strategy, allowing stakeholders the opportunity to efficiently make assessments and provide feedback on design aspects. The web-based map is a valuable tool which enables collaborative discussion in the development of consensus-based design. The design, approach, and application of the interactive web-based map will be presented.

377 Passive Push: A diverless deployment system for passive samplers

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Traditional methods for assessing contaminant bioavailability in sediment risk assessments and remedy performance monitoring generally involve complex and expensive characterization of biota uptake either in the field or in the lab. Recently, passive sampling devices have become more popular for assessing sediment and water contamination; the bioavailable phase of the contaminants sorb to the sampler surface with time in a predictable manner. Detailed calibrations and performance assessments, along with incorporation of performance reference compounds has led to the ability to apply these devices both qualitatively and quantitatively in the field and the laboratory as evidenced by Environmental Protection Agency (EPA) endorsement. As the technology transitions to application, one of the key limitations that has been identified is the ability to reliably deploy and retrieve the devices in the field in a cost effective manner. Sediment remediation in Pearl Harbor requires long-term monitoring, which provided an opportunity to conduct a large-scale test of a new passive sampler deployment system, while also providing valuable data to the Pearl Harbor sediment remediation program. The Passive Push system was developed to deploy passive samplers using one of two methods: a customized drive frame and a push pole system. Sampler frames were built to accommodate different types of passive samplers, but for this project polyethylene samplers were used to target PCB contamination. Samplers, sandwiched in frames, were installed such that a portion of the sampler was buried in the sediment and a portion was exposed to the overlying water, to provide information on bioavailable contaminants in both pore waters and seawater above the sediment interface. Timed-release buoys were used for automated retrieval 35 days after sampler deployment. Both sampler deployment systems were tested, and samplers were installed from a small survey boat at 24 stations throughout the entire Harbor. Traditional sediment and fish-tissue samples were also collected from those stations as part of the long term monitoring program. The Passive Push deployment and retrieval system demonstrated better sampler deployment efficiency and better recovery success than traditional methods using SCUBA. Deployment methodology and results from the Pearl Harbor sampling will be presented.

378 Assessing remediation of contaminated sediments on the Ottawa River using chemical and biological endpoints

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The Ottawa River is a component of the Maumee, Ohio Area of Concern (AOC) as defined by Great Lakes Water Quality Agreement between the U.S. and Canada. A Great Lakes Legacy Act sediment remediation project took place in the lower 14.2 km of the river where urban and industrial activities impacted the beneficial uses of the river. As a potential remedy sediment was removed by dredging based on a surface weighted average concentration model where PCB and PAH levels exceeded targeted levels. Each year from 2009-2013 and again in 2015, various chemical and biological indicators were collected to assess the remedy effectiveness: tissue concentrations in fish from different trophic levels (largemouth bass, brown bullhead, white sucker, pumpkinseed, gizzard shad, bluntnose minnow, emerald shiner), macroinvertebrates (collected with multi-plate samplers) and tetragnathid spiders. DNA damage was measured in brown bullhead blood. Macroinvertebrate biotic condition was assessed, primarily using Ohio's multimetric Lacustrine Index of Community Integrity (LICI). DNA damage in brown bullhead increased during dredging then declined in subsequent years. Based upon one measure of biotic integrity (LICI), no difference was found pre-and post-dredge, though other metrics/ analyses will be explored. Unlike PCBs, whose concentration decreased in Gizzard Shad, Emerald Shiners, Bluntnose Minnows, Largemouth Bass, macroinvertebrate and spider tissue levels 5years post-dredge, in most media, there was no significant change in PAH concentrations. For example, levels of total priority PAHs did not change pre- and 5 years post-dredge for PAHS in water, sediment, brown bullheads, emerald shiners. Levels of PAHS in macroinvertebrates were lower pre-dredge, and levels of PAHs in bluntnose minnow were lower 5 years post-dredge. A likely contributing factor to the contaminant-specific results is that, at this site PCBs are mostly legacy contaminants, while there are nearby sources that can still be introducing PAHs into the river in significant amounts.

379 Use of Recretohalophytes to Remediate Salt-Impacted Sois

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Increasingly, salt-impacted soils are becoming an environmental issue affecting both global soil quality and plant productivity. Cement kiln dust (CKD) is a fine-grained, solid, highly saline waste product of the cement manufacturing process. CKD was landfilled at a site in Bath, ON, Canada over a 30-year period resulting in a wasteland largely devoid of vegetation. Phytoremediation techniques were investigated to remove salt from the site, and prevent salt runoff into Lake Ontario. In previous work, *Phragmites australis* (a resident species) was found to phytoextract large quantities of the dominant salt ions, K^+ and Cl^-. Although phytoextraction with proved very effective at this site (with a potential to remediate it in 7-9 years), due to the invasive variety of *P. australis*, this phytoextraction work cannot be applied to the hundreds of other CKD site in Canada. Hence, two native, halophytic grasses, *Distichlis spicata* and *Spartina* *pectinata* were identified as having potential to remediate the soil via excretion and subsequent haloconduction. During this process, salt is transported via the xylem into plant biomass, excreted through specialized salt glands onto the plant leaf surfaces, and then dispersed by the wind. Analytical work using X-ray dispersive scanning electron microscopy was carried out to identify chemical and physical properties of the salt crystals. Multiple trials were carried out both in the field, and under controlled conditions in the greenhouse in order to determine the most efficient way to collect dispersed salt particles over varying distances. These data are being used to model salt dispersion patterns over space and time, in order to determine the effectiveness to remediate salt-impacted soils using recretohalophytes.

380 Evolution of Water Chemistry During Recovery of a Mining Impacted Waterway in Black Hawk, CO

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The legacy impacts of hardrock mining are complex and difficult to fully mitigate. These impacts are especially widespread in Colorado, which features at least 10,000 of the nearly 53,000 abandoned mine lands registered with the Bureau of Land Management as of January 2017. Though the effects of hardrock mining on water quality have been extensively researched, there is a paucity of data documenting the real-time responses of mining impacted waterways to remediation efforts in the literature. In seeking to breach this informational gap, we collected monthly water samples at 7 different locations along the mining impacted North Fork of Clear Creek (NFCC) beginning west of Black Hawk, CO, moving downstream to a recently completed lyme treatment plant, and ending at the confluence of NFCC and the mainstem of Clear Creek. Samples were analyzed for metal and cation concentrations by ICP-AES, anion concentrations by IC, and dissolved and total organic carbon by a Shimadzu TOC-V analyzer. Additionally, we collected field measurements of pH, alkalinity, ferrous, and conductivity. Our analyses have revealed that although pH has remained relatively stable over the duration of the sampling period (a moderate range between 6 and 7.5 for all sites), drastic decreases in both ferrous concentrations (approximately five-fold) and conductivity (approximately three-fold initially, and up to nearly ten-fold at the most recent sample date) were observed immediately following the first treatment period. Metals exhibited a sharp initial decrease and continued decreases over the entire sample period as well. For example, total copper measured ~0.1 mg/l prior to treatment, ~0.06 mg/l following 2 days of treatment, and ~0.02 mg/l following nearly 2 months of treatment. Iron exhibited a similar trend, measuring ~19 mg/l prior to treatment, ~9 mg/l following 2 days of treatment, and ~3 mg/l following nearly 2 months of treatment. The low, but non-zero metals concentrations suggest continued contributions from the untreated AMD, unidentified ground water sources, or the bed sediments. In order to fully capture the response of NFCC to remediation efforts, we are also performing studies of copper bioavailability and sediment chemistry during the recovery process.

381 Performance of Commercially Available Soil Amendments for Enhanced Copper Removal in Bioretention Structures

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While a recent study demonstrated that conventional bioretention media can remove > 90% of copper from copper roof runoff, the median Cu concentrations at the point of discharge from bioretention structures (66 µg L^{-1}) still did not achieve the lowest copper levels in stormwater discharges sought by some jurisdictions (< 10 µg L^{-1}). In laboratory column studies, commercially available soil amendments, Biochar, Greensand, and Zeolite, were evaluated for their ability to enhance the performance of bioretention media for copper removal. Pilot column studies demonstrated their ability to increase copper attenuation when used as amendments. In a more extensive column study, each soil amendment was tested in duplicate as underlayer and admixture treatments along with control columns containing standard bioretention media. Artificial stormwater containing 4,000 µg L^{-1} Cu was added to each column in 2.00 L increments using a peristaltic pump at a 10 cm^3/hr flow rate to simulate maximum concentration and event volume conditions exhibited in the previous field study. The most effective amendment-treatment combinations, based on copper exports over bed volumes of copper spiked stormwater added, were selected for further testing. A similar, more intensive, column study was performed for the chosen combinations where the media in each column was halved, and the artificial storm water copper concentration was increased to 20,000 µg L^{-1}. These column studies demonstrated that amended columns can consistently export < 10 µg L^{-1} Cu during copper loading similar and more extensive than natural conditions. The Zeolite Underlayer treatment consistently exported lower copper concentrations compared to all other columns, including control, and was the last column to experience breakthrough; a point where the binding capacity of the medium in the column appeared to reach saturation, and copper exports substantially increased from previous equilibrium state. Zeolite as an underlayer was chosen for use in an ongoing field study where copper roof runoff is treated by bioretention planter boxes. Copper roofing runoff is treated in duplicate planter boxes containing standard bioretention media or duplicate planter boxes containing standard bioretention media and amended with a zeolite underlayer. Results of the field study evaluating the performance of the zeolite amended planter boxes compared to standard bioretention media planter boxes will also be presented.

382 Introducing NewTAML Activators for Ultra-Dilute Catalytic Oxidation in Global Water Treatment

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TAML activators, a unique class of iron-centered oxidation-resistant small molecule enzyme mimics, present a solution for removing organic micropollutants during water purification. Here we present NewTAMLs, a new iteration of TAML activators featuring sulfonamide-containing ligands comprised entirely of biochemically common elements. In studies with model substrates, NewTAML/peroxide treatment delivers optimal performance at the ideal pH range of 7–8, significantly outperforming older TAMLs in the breakdown of persistent substrates. In pre-pubertal mice, NewTAML/peroxide pretreatment of ethinylestradiol-laced drinking water results in a dose-dependent reduction in uterotropic response with no toxic effects. With exceptional activity, non-persistence, non-toxicity, and low cost of production, NewTAML processes represent a potential breakthrough for global water treatment of organic micropollutants.

Canadian Oil Sands – Advancing Science in Chemical and Toxicological Characterization, Reclamation and Monitoring

383 In situ biodegradation of naphthenic acids in groundwater near oil sands tailings ponds

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Potential seepage of naphthenic acids (NAs) from tailings ponds into surface water and groundwater is one of the main environmental concerns associated with oil sands mining operations. While a growing number of studies have demonstrated the microbial breakdown of NAs under both aerobic and anaerobic conditions in controlled laboratory experiments, the direct verification of in situ biodegradation of NAs in impacted groundwater remains a challenge. Here we report the application of ^{13}C-labelled

NA surrogate compounds to evaluate intrinsic biodegradation of NAs along two separate groundwater flow-paths originating from two different major oil sands tailings ponds. Microcosms containing the labelled NA surrogates (cyclohexanecarboxylic acid, 1,2- cyclohexanedicarboxylic acid and 1-adamantanecarboxylic acid) were lowered into monitoring wells for several months to allow sufficient time for substrate degradation and formation of a biofilm in conditions characteristic of the local aquifer. The subsequent determination of highly ^{13}C-enriched carbon isotope (δ^{13}C) values in phospholipid fatty acids (PLFAs) – biomarkers for the active microbial population – confirmed the microbial breakdown of NAs in the subsurface. Along one groundwater flow-path, where the drive-point well containing the microcosm was situated in an area dominated by muskeg, microbial community analysis revealed a large component of *Dechloromonas aromatic*, an aromatic hydrocarbon-degrading *Betaproteobacteria*. The in situ biodegradation of low molecular weight ($n < 12$) NAs demonstrates that these compounds can be readily broken down by the indigenous microbial population found in the shallow sub-surface near tailings ponds. Since many low molecular weight NAs are considered some of the most harmful compounds in oil sands process-affected water (OSPW), this study has shown that a reduction in OSPW toxicity can occur in impacted groundwater prior to surface discharge.

384 Reproductive and developmental disruption in wood frogs following acute exposures to acid extractable organics from oil sands-process affected water

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Oil sands process-affected water (OSPW) is produced as a result of bitumen extraction in the Canadian Oil Sands region. The OSPW is stored in tailings containment structures to permit water re-use and because discharged to surface water is not approved under current regulations. OSPW contains many compounds, although naphthenic acids (NAs) have been identified to be the primary toxic constituent to fish. We have undertaken studies to determine the effects of NAs from OSPW on reproductive health and development in wood frogs (*L. sylvaticus*). Wood frogs are widespread in North America, including the oil sands region of Alberta. We developed a novel exposure regime for wood frogs under controlled laboratory conditions. With this approach, we are able to monitor spawning, fertilization and early larval development. For our exposures, naïve frogs from both sexes were caught in Ontario, Canada in April 2016 and 2017 during spring spawning. Frogs were then divided up into 4 treatment groups (n=15 couples per treatment group) consisting of 0 (control), 3, 10 and 30 mg L^{-1} nominal concentration of OSPW NA extracts. Males and females were exposed separately for 24h. Thereafter, they were placed together in tanks conducive for breeding and observed every 20 min for 60h. Results were consistent in both years. The OSPW NA extract (10 and 30 mg L^{-1}) disrupted spawning behaviour, reducing the occurrence and time in amplexus, and time to oviposition. All eggs laid by frogs exposed to 10 and 30 mg L^{-1} developed abnormally, with asynchronous cell division and blastodermal edema resulting in mortality. Fertilization rates were reduced only at 30 mg L^{-1} where less than 10% of egg masses were viable. These data indicate that NAs found in OSPW inhibit wood frog reproduction at concentrations $\geq$ 10 mg L^{-1}. Therefore, these NA concentrations should be considered in the development of treatment targets for OSPW.

385 Effects of parental embryonic exposure to raw and ozonated oil sands process-affected water on second-generation zebrafish

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Oil sands process-affected water (OSPW), a by-product of the oil sands mining industry, is stored in on-site tailings ponds. To increase OSPW quality and permit its integration back into the environment, treatment, both passive and active, is currently required. Ozonation is currently being studied as a possible tool for the treatment of OSPW. In this study, zebrafish embryos were exposed to both 100% raw and ozonated OSPW from 0-7 days post fertilization (dpf). Survival was monitored and gene expression and DNA methylation were measured at 7dpf. Exposed embryos were then grown to adulthood in clean water. These fish were bred and their embryos were collected and reared in either clean water (unexposed second-generation) or the same OSPW type that their parents were exposed to (exposed second-generation). Breeding success of the first-generation developmentally exposed fish was determined by measuring number of pairs that spawned, fertilization rate, and survival of unexposed offspring. Gene expression and DNA methylation were also measured in 7dpf offspring. Developmental OSPW exposure did not affect breeding success of first-generation zebrafish. Raw OSPW exposure induced the expression of biotransformation genes cytochrome P450 1a and 1b in first-generation 7dpf embryos, while ozonated OSPW did not. Exposed second-generation embryos had a very similar response in expression of these biotransformation genes as the first-generation, while unexposed second-generation embryos showed no change in expression. However, unexposed second-generation embryos did show increased expression in some genes. This indicates a change in basal gene expression, which could potentially be due to changes in DNA methylation. Global DNA methylation was not affected by OSPW exposure in first-generation embryos. However, unexposed second-generation offspring from parents exposed to raw OSPW had a trend towards decreased global DNA methylation. Understanding what changes in DNA methylation mean for survival outcomes for wild fish populations will require further study. Overall, it appears that developmental OSPW exposure has little effect on second-generation embryos. Though second-generation endpoints are often overlooked, they are important to consider when evaluating the overall risk of OSPW exposure.

386 Examining reproductive effects of dilute bitumen exposure in wild fathead minnows from the IISD-Experimental Lakes Area

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The International Institute for Sustainable Development-Experimental Lakes Area (IISD-ELA) is embarking on a comprehensive program of study to examine the fate, behaviour and potential impacts of diluted bitumen (dilbit) in the freshwater aquatic environment as well as strategies to enhance recovery of impacted freshwater ecosystems. During the 2017 field season, baseline data and initial studies to refine methods were conducted in preparation for model spill studies in subsequent years. Here we report initial results from exposures of wild adult fathead minnows (FHM) (*Pimephales promelas*), captured from lakes at the IISD-ELA, to dilute concentrations of dilbit water accommodated fractions (WAFs). Adult FHM (n=50m/100f) were exposed to high energy mixing WAFs at concentrations of 1:100,000 or 1:1,000,000 for 21 days. An unexposed reference group was also included. After the exposure period, FHM from each treatment were relocated to 10L breeding chamber aquaria so that 1 male and 2 females from a treatment were housed together in a breeding triplet (n=12 triplets per treatment) and ambient temperature was incrementally raised to 22°C to induce spawning activity. Video analysis of time spent in the breeding area and total number of spawning events was used to determine reproductive activity, and reproductive output was determined by the total number of eggs produced over a 14 day breeding period. Ongoing analysis of egg diameter, hatching success, and mean larval length and weight at landmark developmental time points will be used to determine potential effects of exposure to the dilute WAFs among progeny of exposed FHM.

387 Toxicity testing of oil sands process waters: Industry needs and data gaps

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Recent international attention on Alberta's oil sands has increased the rate of scientific publication 10-fold, and in recent years almost 25% of environmental impact studies relate to the toxicity or chemistry of oil sands process-affected waters (OSPW), although the waters are not currently released. The oil sands industry has historically followed a no-release practice for OSPW, which has resulted in a significant accumulation of stored waters used for process recycling. Over 650 articles relating to OSPW toxicity have been published to date, with 500 of those articles published in the last 4 years (2013-2017). Much of the research fails to properly identify the waters tested, uses novel test organisms or endpoints, or fails to meet criteria needed to formally evaluate potential toxicity. Of the 500 articles surveyed, only 45 sought to characterize OSPW toxicity with established toxicological protocols. In these papers, the fraction of OSPW tested, the manner, in which the OSPW was handled, and the biological response levels evaluated through OSPW exposure vary greatly. Research into water quality and toxicological properties of OSPW needs to see a shift from answering broad un-focused questions towards promotion of identification of specific target constituents and establishment of treatment priorities. It is hoped that the formal definition of research needs and gaps will improve research relevance and support decisions relating to evaluating the potential risks for water return.

388 Application of the target lipid model and passive samplers to characterize the toxicity of the organics in oil sands process-affected water

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Sustainable oil sand operations will eventually include the return of treated process-affected waters to the environment. This requires the development of water quality benchmarks for the organic constituents in the oil sand process-affected water (OSPW) to ensure potential risks to receiving waters are managed. Assessing the toxicity and risk of mixtures of organic constituents in OSPW is complicated by the concentrations of different nonpolar and ionized compound classes, which can vary temporally and spatially and the lack of standard analytical methods. To address this challenge, passive samplers were employed to estimate the total concentrations of bioavailable organics in OSPW using biomimetic solid phase extraction (BE) with the aim to predict toxic potencies of these difficult to characterize mixtures. This method was optimized by conducting toxicity tests on a series of model compounds, individually and in defined mixtures, as well as with mixtures of acid extractable organics from OSPW for several fish and invertebrate species. The results of this work establish a successful proof of concept demonstrating that passive samplers are a rapid and convenient analytical technique that can be applied to characterize the toxicity of OSPW thereby supporting further efforts to develop BE-based water quality benchmarks.

389 Base Mine Lake 2038: Using analytical chemistry to predict the future of oil sands end pit lakes

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An in-depth review of the organic profiles of oil sands process-affected water (OSPW) from Syncrude Canada Limited has been completed using high pressure liquid chromatography-ultra high resolution Orbitrap mass spectrometry in both positive and negative mode, here, for the first time. Three types of OSPW were compared in an attempt to identify the most persistent chemical classes: 'aged' samples from three experimental ponds (at least 25 years old) were used as surrogate projections of future OSPW. 'Fresh' OSPW samples (4) were taken from active tailings ponds. Additionally, surface water sampled in 2014 from the first oil sands end pit lake (Base Mine Lake, BML) was also analyzed. Principal components analysis was able to distinguish OSPW types by relative contribution of acidic chemical classes (negative mode). Fewer species were detected in aged OSPW samples, and the oldest samples had the lowest intensities (concentrations) of organics. Oxygen-containing acids (including naphthenic acids (NAs), O_2^- species) dominated the profile of fresh OSPW whereas in aged OSPW, the mono-oxygenated naphthenic acids (O_3^- species) and NAs had similar relative contributions. Dissolved organic compounds detected in positive mode may have slower rates of degradation than their acidic counterparts, with O_2^+ demonstrating the least variation in concentrations across all OSPW types, suggesting this to be a persistent class. Chromatography, however, revealed that many O_2^+ species include two or more distinct unresolved isomer groups- some of which are completely absent in aged OSPW. Nitrogen and sulfur-containing organics were nearly absent from the profiles of aged OSPW. Lower concentrations of total organics in aged OSPW may indicate that BML OSPW will become less toxic in the future.

390 Investigation of oil sands groundwater quality and a case for their release to surface water receiving environment

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The chemistry, and acute and chronic toxicity of groundwaters associated with Shell Canada's Albian Sands operations in northern Alberta were characterized as part of an effort to develop a burden of evidence that the release of groundwaters would pose a negligible and understandable risk to a receiving aquatic environment. Concentrations of conventional chemicals such as nutrients and metals were generally below concentrations considered to pose a negligible risk of harm in long-term exposures. Concentrations of naphthenic acids were variable, due in part to the analytical method employed, but routinely in excess of 1 mg/L. Groundwaters rarely caused lethality to fish and invertebrates in acute toxicity tests. The oil sands groundwaters caused similar or less sublethal toxicity as has historically been recently produced by effluents regulated by the Pulp and Paper Effluent Regulations or the Metal Mining Effluent Regulations. These groundwaters have somewhat high oxygen demand associated with nutrients and organic substances, and therefore have the potential to enrich receiving surface water environments if untreated and depending on ratios of mixing with a receiving environment.

Multiple Stressors I – Approaches for Deciphering Multiple Stressors in Aquatic Environments

391 Metabarcoding and Aquatic Bacteria in Streams: What can microbiology tell us about stream stressors?

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The microbiotic community in a stream is a complex assemblage of bacteria, phytoplankton, fungi etc. which respond to and interact with external stressors, including stream chemistry, land use, nutrients, and contaminants. The composition, diversity and function of these microorganisms may be an important indicator of stream health and function. Metabarcoding high throughput sequencing methods are becoming increasingly accessible and affordable, and present an opportunity to develop new methods of evaluating ecosystem condition and response. We used standard barcodes to amplify the 16S ribosomal RNA region and identify over 30,000 bacteria species in water samples collected from NH streams. Samples were collected at 40 sites in coordination with the NH Department of Environmental Services River Trend Monitoring and Volunteer River Monitoring Programs. Samples collected in June, July and August 2016 were extracted at the UNH Genome Center, and analyzed on an Illumina Platform. The NHDES program also collected data on water chemistry, land use and macro invertebrates at each site. We applied multivariate and phylogenic network analyses to identify correlations between community structure and water quality parameters, and found that stream microbiology responds to both water quality stressors (e.g. dissolved oxygen, nutrients) and land use. These results indicate that microbial assemblages respond predictably to multiple stressors, and may be useful as indicators of biologic condition in aquatic systems.

392 Impaired mitochondrial function in zebrafish exposed to hypoxia and polycyclic aromatic hydrocarbons

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Aquatic environments worldwide face degrading ecosystems due to anthropogenic and environmental stressors. Many estuaries are plagued with seasonal hypoxia, a result of both natural processes and anthropogenic pressures. These same estuaries can also be highly contaminated with pollutants such as polycyclic aromatic hydrocarbons (PAHs), known carcinogens and teratogens. Embryonic co-exposures of PAHs and hypoxia result in synergistic toxicity through mechanisms that have not been fully elucidated. Based on reports of hypoxia and PAHs altering mitochondrial function and integrity, we hypothesized that mitochondrial dysfunction could be a mechanism underlying the synergistic toxicity seen during co-exposures. To test this, we exposed zebrafish embryos to control conditions, hypoxia, a complex mixture of PAHs, or a combination of hypoxia and the PAH mixture. After 24 hours of exposure, embryos were monitored for oxygen consumption rate (OCR) in the Seahorse Extracellular Flux Analyzer (Agilent Technologies). Initially, basal OCR measurements were taken over a 4-hour period. Based on these results, we chose three recovery time points, 45 minutes, 5 hours, and 18 hours, for further bioenergetics testing. Using pharmacological agents, we assessed embryos for several functional parameters, including maximal respiration, oxygen consumption due to ATP production, spare capacity, and proton leak. Hypoxia exposure, with and without concurrent PAH exposures, resulted in significantly lowered basal mitochondrial respiration, non-mitochondrial respiration, and ATP production (all $p< 0.05$). However, hypoxia exposure did not alter the maximal respiration of these embryos, which resulted in significantly increased spare capacities ($p< 0.05$). PAH exposure significantly altered OCR due to proton leak within embryos ($p< 0.05$). Given sufficient recovery periods, basal mitochondrial and maximal mitochondrial respiration of exposed embryos returned to levels indistinguishable from control embryos. However, there were still significant effects of PAH and hypoxia exposures on proton leak, OCR due to ATP production, and non-mitochondrial respiration after 18 hours of recovery. Our data suggest that synergistic mitochondrial toxicity is not the main cause of the exacerbated toxicity seen during PAH and hypoxia co-expsoures. However, the drastic effects hypoxia has on mitochondrial function could impair an organism's ability to respond appropriately during PAH exposures.

393 Evaluating relations between stressors and ecological endpoints in streams at the regional scale

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Understanding the relative importance of multiple stressors on biological condition requires extensive physical, chemical, and biological data and statistical modeling. Each year during 2013–17, the U.S. Geological Survey conducted an intensive multi-stressor assessment of small streams in a different, geographically diverse region of the United States. Water quality was sampled weekly at about 100 wadeable streams in each region during the spring/summer growing season, and continuous stage and temperature were measured at all sites. This 6–12 week index period culminated with a habitat and biological survey of each stream and bed-sediment collection for chemical analysis and toxicity testing. About 1,000 chemical constituents were measured in water and sediment samples. The data are being used to evaluate the effects of multiple chemical and physical stressors on algal, invertebrate, and fish communities. Results from the Midwest Stream Quality Assessment (MSQA), done in 2013, were analyzed using classification tree analysis (fish) and boosted regression tree models (algae and invertebrates). The models indicate that stressors from all major categories—habitat, nutrients, and contaminants (primarily pesticides)—are affecting biota. Specific stressors found to be significant varied depending on taxon group and, within each taxon group, community metric. These results likely will differ from those of other regions because of major differences in geography, in particular, land use; the Midwest is heavily agricultural and the other four regions are extensively urbanized.

394 Toxicological Effects of Diltiazem in Larval Fathead Minnows (Pimephales promelas) across Dissolved Oxygen Gradients

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Water resources in arid to semi-arid regions of the United States are stressed by population growth and drought especially in urban areas where flows can be dominated by or even dependent on wastewater treatment plant discharges. In these urbanizing watersheds, fish are exposed to multiple stressors such as contaminants of emerging concern (e.g., pharmaceuticals) and hypoxia. The impacts to fish following exposures to both chemical (e.g., pharmaceuticals) and nonchemical (e.g., dissolved oxygen) stressors have received little attention when urbanization and the occurrence of hypoxia is increasing worldwide in both coastal marine and freshwater ecosystems. Recent field observations from our group identified concentrations of diltiazem (DZM), a vasodilator used to treat high blood pressure, in fish plasma exceeding human therapeutic doses (e.g., C_{max}) along multiple Texas gulf coast estuaries which are commonly listed on the Texas 303(d) list for non-attainment of dissolved oxygen (DO) water quality criteria. The objectives of the present studies were to determine (1) the toxicological effects of diltiazem and DO singularly and (2) whether diltiazem differentially affects larval *Pimephales promelas* across DO gradients following acute and chronic exposures using standard and alternative endpoints. *P. promelas* mortality increased across DZM concentrations and decreasing DO levels (e.g., DO 8.2 mg/L LC_{50} = 28.2 mg/L DZM; DO 5.0 mg/L LC_{50} = 16.0 mg/L DZM; DO 3.0 mg/L LC_{50} = 8.3 mg/L DZM). Diltiazem concentrations studied at 3 mg/L and not 5 mg/L DO resulted in significant interactive effects which were observed across all endpoints (e.g., mortality, growth, heart rate, and behavior). Other alternative endpoints investigating the physiological effects of diltiazem on larval fish cardiac function (e.g., contractility, stroke volume) were twice as sensitive as mortality following acute exposures (48 h). Interestingly, preliminary larval fish studies showed significant effects at 15 mg/L DZM and 8.2 mg/L DO on atrium contractility and ejection volume while no effects were observed on the ventricle. This presentation will discuss our toxicological and physiological results following laboratory studies on DZM and DO singularly, and the DO x DZM co-exposure studies indicating an underestimation of environmental hazards to fish under realistic exposure scenarios to multiple stressors.

395 Bioaccumulation and ecological effects of contaminant stressors in Columbia River aquatic food webs

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Effluent containing complex mixtures of contaminants of emerging concern (CECs) is discharged to the Columbia River. Some CECs accumulate in bed sediments and may impair aquatic ecosystems. In recent years, several interdisciplinary studies in the Columbia River Basin have assessed the effects of different classes of CECs and traditional bioaccumulative compounds in the food web. The goal of these studies is to determine whether exposure to and uptake of these contaminants are linked to detrimental biological endpoints in fish and wildlife. Organisms studied include species of ecological and cultural importance such as largescale suckers (*Catostomus macrocheilus*), Pacific lampreys (*Entosphenus tridentatus*), white sturgeon (*Acipenser transmontanus*), and osprey (*Pandion haliaetus*). Contaminant levels are compared between species in the context of the trophic level of the organism and chemical properties of the compounds to improve understanding of distributions, bioaccumulation, trophic transfer, and effects of contaminants on key species and food webs. In this presentation, special focus will be given to the indigenous Pacific lamprey (not to be confused with the invasive sea lamprey), an ancient and poorly understood species that plays an important ecological role over a very large range and has deep cultural significance to Native Peoples in the Pacific Northwest. Especially relevant to this session is the fact that Pacific lampreys are an integrator of multiple stressors – habitat loss, dam passage, climate change, and water quality impairments. Pacific lampreys are affected by all of these issues and no single stressor fully explains their decline. Prior to our studies, almost no data existed on contaminant bio-burdens in Pacific lampreys. However, we recently measured flame retardants, pesticides, and industrial and personal care compounds at concentrations of concern in larval and adult Pacific lamprey tissues. We suspect that contaminants are contributing stressors that aggravate the effects of known major stressors such as dam passage and habitat loss implicated in the precipitous reduction in returns of this species.

396 Patterns and ecological influences on coal combustion residual (CCR) distribution in freshwater lakes

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Coal combustion residuals (CCRs) make up the second largest waste stream in the United States. As a result of their continuous disposal, CCRs represent chronic sources of trace element stressors (i.e. Se, Cu, Zn) to freshwater lake ecosystems where they accumulate in sediments and distribute among environmental compartments on a site-specific basis. In the present study, we track the movement of CCR-derived trace elements from abiotic compartments (surface water, sediment, and sediment pore water) to biotic compartments (biofilm, zooplankton, and three fish species) in Sutton Lake, a hydrologically closed lake system in Wilmington, NC. In order to treat samples as a function of their entire trace element profile, rather than assess elements one-by-one, we use principal components analysis with PERMANOVA and a variety of secondary analyses to ask "what factors account for differential trace element uptake and transfer through aquatic food webs?" Results are initially compared to a non-CCR receiving reference lake and subsequently with to additional lakes with similar CCR-loading but differing limnology and biogeochemistry. We report that CCR distribution not only varies by compartment ($p < 0.001$), but patterns of distribution vary among pairs of lakes ($p < 0.017$). In addition, we report differential CCR bioaccumulation by fish species ($p < 0.001$) which may be explained by ecological variables including dietary patterns. Last, we compare field-caught fish data with the results of a lab-based feeding study using field-collected biofilm and zooplankton samples to tease apart the effect of diet on trace element tissue patterns along aquatic food webs. In addition to informing resource managers about the influence of these waste streams on freshwater ecosystems, these data can guide environmental monitoring plans as well as decisions concerning remediation efforts now that coal ash basins are being retired in the southeastern United States.

397 Contrasting predictors of acute toxicity and benthic community response to marine sediment contaminants in Southern California

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Previous research into chemical contaminants' environmental impacts often focused on one chemical at a time. The impacts of mixtures of chemicals in marine sediments on organisms and on ecological communities remain poorly understood. The objective of this analysis was to contrast sets of chemicals most associated with acute organismal toxicity versus those most associated with differences in benthic ecology (i.e., macrofaunal abundance, species richness, and indicator species presence) in Southern California. Sediment quality data collected in 2003-2013 from Southern California bays and estuaries were analyzed. Acute toxicity of surface sediments (upper 5 cm) was measured by estuarine amphipod (*Eohaustorius estuarius*) mortality, categorized as nontoxic (>89% survival), or low (89-82%), moderate (81-59%) or high toxicity (< 59%). Ecological function was measured by a categorical index incorporating data on abundance, species richness, and indicator species presence. These two outcomes' similarity was measured by weighted κ, and predictors for each outcome were identified by classification trees based on Gini or mean-decrease-in-accuracy (MDA) criteria, using the R package. The weighted κ for the two outcomes was 0.14 (95% CI: 0.09, 0.19), demonstrating dissimilarity between the benthic community and acute toxicity responses. The most-to-least important chemicals as determined by Gini for benthic community impacts were Cu, Zn, Pb, Hg, and Ni; for the same outcome, the most important chemicals by considering MDA were Cu, Zn, Pb, Ni and Hg. For amphipod mortality, the most-to-least important chemicals as determined by Gini were Cd, Se, Cu, Ni, and Hg; or as determined by MDA: Cd, Hg, Cu, phenanthrene, and Pb. Conclusions: These measures of acute organismal and biological community response reflected distinct biological processes, evinced by their low kappa.Nonetheless, both acute and chronic toxicity share several predictors including Cu and Ni. It is unlikely that these descriptive models represent causal relationships, as metal bioavailabity and toxicity in amphipod toxicity tests is extremely low. Rather, these chemicals might reflect gradients in combined chemical exposure, source, or bioavailability. The chemicals identified as important predictors might be good to investigate further in experimental models for joint acute toxicity, and considered jointly in observational ecotoxicological studies in other settings.

398 Deciphering multiple stressors in an urbanized watershed using statistical and diagnostic tools

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The San Diego River watershed in California is subject to multiple stressors, including altered hydrology, treated wastewater discharges, and various pollutants from urban and agricultural runoff. Previous assessments indicated that high temperature, pyrethroids, nutrients, conductivity, metals, and channelization may be causing observed biological impairment in the lower reach. Using a modified CADDIS framework we evaluated the relative contribution of different stressors at a sentinel site using a combination of statistical and diagnostic tools developed for this region. Using a propensity score approach we identified sites that matched our test site in terms of land uses and other factors, but where a specific stressor of concern was sufficiently low that it was unlikely to be a cause of impairment. This analysis indicated that high conductivity was a key stressor at the site. Evaluation of potential stressors in relation to comparator sites identified using principal components and clustering indicated that temperature, conductivity, pyrethroids, and nutrients were high and possible candidate causes of observed impairment. Stressor-response analyses indicated that conductivity was a key stressor and a Random Forest Model indicated that biological condition had a high probability of being impaired above a conductivity of 1,500 µS/cm. Species sensitivity distribution analyses indicated that temperature, metals, and pyrethroids were unlikely to be a major cause of observed impairment. However, analyses of macroinvertebrate and algal metrics indicated that high nutrients and certain habitat limitations (reduced flood plain) are secondary factors affecting the biota. Taxa tolerance values developed for this region also supported nutrients as a secondary cause. The combination of taxa tolerance information and stressor-response analysis was particularly helpful in distinguishing the relative effect of different stressors.

Environmental Chemistry – Part 1

399 Nuclear Magnetic Resonance Studies of Interactions Between Organic Contaminants and Soil Organic Matter

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Soil organic matter(SOM) plays a key role in the retention, transport, and availability of many organic contaminants in the environment. While this functionality of SOM is commonly modeled as being analogous to a simple partitioning process driven by contaminant hydrophobicity, there is a general consensus that the underlying molecular-level mechanisms are more complex than simple partitioning alone accounts for. As such, the partitioning model for the sorption of many classes of organic compounds into SOM often fails to provide a mechanistic explanation for non-ideal behaviors that impact the fate of sorbed contaminants in the environment, including sequestration, reduced bioavailability, and reduced apparent ecotoxicity. This is particularly true for polar and ionic compounds. Nuclear Magnetic Resonance (NMR) spectroscopy is emerging as a powerful tool to help elucidate the molecular-level nature of the interactions that occur between organic contaminants and SOM. By focusing on the use of organofluorine compounds as model contaminants, multinuclear $^{1}H/^{19}F$ NMR experiments are useful for probing many physical and chemical attributes of the intermolecular associations between these model contaminants and both humic substances and whole organic soils. Attributes that can be probed through NMR studies include: the nature of preferred binding sites within SOM, the orientation of the model contaminants during their interaction, and the dynamics of these interactions. This presentation will discuss the use of NMR spectroscopy to elucidate the roles that the physical and chemical structure of SOM plays in the interactions with model organic contaminants, and that may, in turn, play a role in governing the overall fate and behaviour of these compounds in the environment.

400 Photo-production of triplet excited states in effluent organic matter

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Wastewater effluents are an important component of streams and rivers, especially in urban areas. The dissolved organic matter (DOM) present will be a combination of that occurring naturally and that input from the wastewater effluent. DOM produces photochemically produced reactive intermediates (PPRIs), such as triplet excited state DOM, hydroxyl radical, carbonate radical, and singlet oxygen when exposed to sunlight. These PPRIs can react with organic pollutants and limit their buildup in surface waters. Thus effluent organic matter (EfOM) will play an important role in the fate of wastewater-associated contaminants through indirect photolysis, and it is currently not fully understood how EfOM compares to natural organic matter in terms of PPRI production. To determine the rate at which EfOM produces triplet excited states, effluent waters were collected from sixteen wastewater treatment plants around the state of Minnesota, including five major metro plants serving the Twin Cities, as well as two effluent-dominated rivers (the Santa Cruz River in Arizona and the Santa Ana River in California). 2,4,6-Trimethylphenol was used as a probe to monitor triplet excited state production. Triplet production appears to have no strong correlation to season or wastewater treatment plant. There is a loose correlation between E2/E3 values and triplet production. While some plants had lower triplet production rates than Pony Lake Fulvic Acid, others had greater triplet production rates. These results show that contaminants in waterways which receive a significant amount of effluent flow may degrade at a different rate than those in purely natural waters.

401 Kinetics and Degradation Mechanism of Benzotriazole During UV/Chlorination Process

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Benzotriazole (BTA) is widely used as a corrosion inhibitor of yellow metals, antifreezes, cutting fluids, and coating materials in various industries or even in domestic products. Because of its high polarity and low biodegradability, BTA is expected to be mobile in the aquatic environment. The reported removal of BTA ranges between 29 and 58% in wastewater treatment plant (Reemtsma et al., 2010). In this study, the removal kinetics and degradation mechanisms of BTA during UV/Chlorine process were investigated, especially focusing on UV-A/chlorine process. The experiment was performed with batch type photo reactor. The light intensity of UV lamps (UV-A, B, C) used were 3.3-4.3mW/cm^2. LC-MS/MS was used for BTA analysis. The result showed that the removal rate of BTA was in the order of UV-A < UV-B < UV-C. UV-A/Chlorine process showed a synergetic effect compared to UV-A photolysis and chlorination only processes. The synergetic effect is due to the OH radical generated in the UV/Chlorine process. The kinetics followed the pseudo- first order kinetics. More chlorine dosage, faster removal rate was achieved. Alkaline pH increased the removal of BTA UV-A/Chlorine process. Additionally, transformation byproducts during UV/Chlorine process were identified; using the identified byproducts we proposed the degradation pathway of BTA during UV-A/Chlorine process.

402 Evidence for redox processing of soil sulfur in well-aerated forested catchments of the Great Smoky Mountains National Park

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Sulfur is transformed within the Great Smoky Mountains (GRSM) by numerous biogeochemical processes. Sulfur in GRSM catchments originates from atmospheric deposition and mineral bedrock components, and exists as numerous chemical species in most ecosystem compartments. Results from soil studies, using traditional chemical analysis and stable isotope ($\delta^{34}S$ and $\delta^{18}O$) analysis, evidence dynamic biogeochemical and redox cycling of sulfur, even in the well-drained and highly aerated environment of ridgeline forested catchments. Soil sulfate budgets show a distinct vertical stratification within the soil profile related to soil composition. Stable isotope analysis revealed (1) a sulfur isotope fractionation process operating on soil sulfate, and (2) a distinct lateral spatial pattern in soil sulfur isotope composition related to bedrock mineralogy. Soil sulfur concentrations show higher organic sulfur in surface horizons and higher sulfate concentrations in lower horizons. Sulfur isotope composition of three soil sulfate pools showed enrichment of ^{32}S (decreasing $\delta^{34}S$ values) progressing from soil solution (soluble) sulfate, to electrostatically-bound sulfate, to chemically-bound sulfate. It suggests that biogenic sulfate reduction (which is selective for ^{32}S) occurs progressively over time, with longer retention times (stronger binding strengths) leading to increased chemical reduction (and retention of ^{32}S) and subsequent reoxidation to sulfate during aeration events (e.g., storm water infiltration). Soil $\delta^{34}S$ values and total soil sulfur mass showed increases with distance from known sulfidic mineral bedrock areas within the GRSM. Results from these and future studies provide Park managers with data to better manage the valuable ecological resources of the Great Smoky Mountains.

403 A Framework for Describing PCB Emissions from Paint

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PCBs are inadvertently created in the manufacturing process of pigments used in commercial paints and other consumer products. Most of the congeners produced through these processes are the most volatile of PCBs: Monochloro- and dichlorobiphenyls associated with pigments have been measured in air worldwide. Although it is possible that the manufacturing process and use of pigment-embedded consumer products contribute to environmental contamination, we hypothesize that use of architectural paint is the major source of these congeners to air. For example, previous work has shown that paint is responsible for 0.000001% of the PCB stocks in Chicago but is responsible for up to 7% of total PCB emissions. This may be due to the fact that paint is spread in thin layers over large surface areas and readily available for volatilization. Here we report an investigation of the emission of PCB congeners from freshly applied pigment. Preliminary results indicate that PCB congeners are released from a pigment sample onto a PUF sampler. The mass of congeners on the PUF increases over time and is independent of drying process. We use this framework to extrapolate small environment data to the city of Chicago and evaluate if paint is the contributing factor to concentrations of PCBs 1, 2, 3, 4, 6, 8, 11, 12/13, and 209.

404 Seasonal trends in chemical composition and oxidative and inflammatory potential of atmospheric particulate matter (PM2.5) from Tehran, Iran

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Airborne particulate matter (PM) is a complex mixture of suspended organic, inorganic and biological contaminants that vary temporally and spatially. Inhalation of urban PM has been linked to respiratory, cardiovascular and metabolic diseases, as well as cancer. However, to date the molecular mechanisms by which PM exerts these adverse health outcomes remain poorly understood. Elucidating the role of specific PM components in mediating the cellular responses, as well as characterizing the mechanisms involved in oxidative and inflammatory reactions implicated in PM-mediated diseases remains of key importance. The current study aimed to evaluate seasonal trends in chemical composition, cellular and a-cellular oxidative potential, as well as inflammatory signaling in Tehran's urban $PM_{2.5}$. A year-long sampling campaign collected 24-hour $PM_{2.5}$ samples at the Sharif University,Tehran, which suffers from serious air pollution and dust storms. Monthly composites were prepared for detailed chemical (SF-ICPMS) and toxicological characterization. The activity of the PM samples for Reactive Oxygen Species (ROS) generation was determined using an acellular DTT assay, which measures the consumption rate of dithiothreitol, and an in vitro alveolar macrophage assay – where intracellular ROS production is quantified using a fluorescent probe, dichlorofluorescein diacetate (DCFH-DA). In addition to the oxidative stress measures, PM-induced pro-inflammatory cytokine (tumor necrosis factor alpha; TNF-α) secretion was measured using Enzyme-Linked Immunosorbent Assay (ELISA). To gain insight into the various pathways (including inflammatory signaling) involved in mediating the PM toxicity, mRNA expression of 28 potential gene targets was evaluated using quantitative reverse transcription polymerase chain reaction (qRT-PCR). Particulate-induced ROS activity as well as TNF-α secretion showed strong seasonal patterns. Several metals (i.e. As, V, Ni and Pb) as well as some organic species (i.e. Levoglucosan, Hopanes and Indeno(1,2,3-cd)pyrene) were strongly correlated with macrophage-based ROS production. The highest level of ROS activity was measured in fall samples, while summer PM induced the lowest ROS levels, but elicited the highest TNF-α secretion. Correlation analysis showed that crustal materials were strongly associated with this TNF-α production, but not with ROS activity. This is the first report of the seasonal toxicological effects of Tehran's $PM_{2.5}$.

405 Occurrence of Volatile Halobenzene Compounds in Ice Cores and Surface Snows from High Elevations on Svalbard

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Since 2005 we have analyzed several organic contaminants in ice cores and surface snow samples from Svalbard. Among the groups of compounds are 15 halobenzenes, including 9 chlorobenzenes, 3 bromobenzenes, pentachloroanisole, and 2 chloromethoxybenzenes. None of these compounds are known to have been used on Svalbard. As a group, these compounds have vapor pressures (VP) higher than any others we have analyzed: The least volatile of the halobenzenes, hexachlorobenzene, has a vapor pressure more than twice as high as PCB70, a common PCB congener with similar molecular mass found in ice cores. The other 14 compounds on our list have VP at least one order of magnitude greater than HCB. In general, these halobenzenes are considered to be too volatile to condense from the atmosphere and accumulate on any surface. However, at least part of the time at high elevation on Svalbard, temperatures are apparently cold enough for these compounds to condense. All of these 15 compounds have been found in at least one sample on Svalbard indicating persistence and long-range transport. Some of them are found in more historic ice core samples, suggesting that they are no longer being emitted from source regions. The most abundant compounds are the DCBs, which are also the most volatile: 1,4-DCBz, is most abundant overall, once used in moth balls, among other things. Its abundance is followed by 1,3-DCBz, and 1,2-DCBz, both known to be used in termite treatment at one time. These compounds are followed in abundance by pentachloroanisole, a methylated byproduct of pentachlorophenol, then by 1,2,4-trichlorobenzene used in herbicides, termite treatments, and as a die carrier. The only other compound with a significant presence is 3,4,5,6-tetrachlorodimethoxybenzene (3,4,5,6-tetrachloroveratrol) which was known to be produced in the bleach Kraft industry by methylation of chloroguiacols, chlorinated analogs of compounds released during delignification by chlorine gas during pulp production processes in the 1970s. Most other chlorobenzenes, including PeCB and HCB, found in lower abundance, were used as intermediates for other chemicals, but produced in large quantities and among compounds on the Stockholm Convention. The appearance of these highly volatile halobenzenes in high concentrations on Svalbard suggests that measurement in the Arctic is necessary.

406 "Barrier effect" of the Himalayas on long-range atmospheric transport of persistent organic pollutants

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Mid-latitude high mountains are considered to be an important role on the global distribution of persistent organic pollutants (POPs) due to their sharp temperature gradients and high precipitation in high altitudes. The semi-volatile POPs transport from lowlands to high altitudes with upslope valley wind, and then deposit driven by low temperature, precipitation scavenging, and forest absorption, which influence the global transport of POPs. The Himalayas is the highest mountains with the average altitude of >5000 m, and the low region near the Himalayas, South Asia, is an important POPs emission region. Recent studies focused on the atmospheric POP transport from South Asia to the Tibetan Plateau driven by Indian monsoon, but few is known about the processes of going through the Himalayas during transport. In the current study, using XAD-2 based passive air samplers (XAD-PAS), we established a sampling transect with altitude of 135-5100 m on southern slope of the central Himalayas, Nepal. Three-year observation were conducted with six-month durations, and soil samples were also collected. The results showed that the concentrations of all the target POPs [such as Dichlorodiphenyltrichloroethanes (DDTs), Hexachlorocyclohexanes (HCHs), Hexachlorobenzene (HCB), and polychlorinated biphenyls (PCBs)] and the compositions of heavier compounds decreased with increasing altitude, indicating that upslope transport is the main pathway of POPs transport from lowland to high altitude. The concentrations of atmospheric POPs in lowland are no significant difference between in monsoon and in non-monsoon seasons, but in the two highest sites (4200 m and 5100 m), higher concentrations were observed in monsoon seasons, suggesting that strong Indian monsoon enhanced the POP transport to high altitudes. In addition, the highest concentrations of soil POPs occurred near the altitude of 2500 m, relating to the "pump" from air to soil by foliage in forest and heavy precipitation in mid-altitude. Fugacity model evaluates that soil in the altitude of < 4500 m is the 'sink' of POPs, and most of POPs deposited on surface ground are stored into soil. Only less than 10% atmospheric POPs transport across the Himalayas to the Tibetan Plateau via relative low-altitude valleys. The results suggested that the Himalayas (and even the Tibetan Plateau) is a barrier to preventing the POPs emitted in South Asia diffuse to globe and decreasing the global influences of South Asia pollution.

Environmental -omics Measurements – Applications to Human and Ecological Exposure and Health

407 Assess personal smoke exposure and acute lung inflammation metabolomics of Exhaled Breath Condensate in fire fighters by NMR-spectroscopy

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Respiratory and cardiovascular causes account for more than half of work-related deaths among fire fighters. Fire fighters comprise the largest group of public safety employees with more than 350,000 career fire fighters and more than 780,000 volunteer fire fighters in the United States. Respiratory protection is afforded to fire fighters responding to structural fires. The prolonged use of respiratory protection apparatuses in wildland fires is very limited due to their weight and limited air capacity. The characterization of smoke exposures and biological (or clinical) monitoring is scarce as a result of the inherent hazards of working in an "*active*" environment interfering with fire fighter's tasks during a fire event.
The purpose of this study is to give information on the dose-response relationship between exposure (smoke and its components) with airway inflammation using Exhaled Breath Condensate (EBC) metabolomics. Collection of EBC by cooling the exhaled breath through a tube is a non-invasive, easy and cost-effective method to collect airways secretions. EBC is a highly concentrated aqueous mixture of many exo- and endogenous chemical species that provide direct information on lung condition. Personal exposures to fine particles were assessed by light-weight pump-operated personal samplers that collect the air near the breathing zone. The chemical composition of fine particles emitted during prescribed burns and EBC samples taken before and after prescribed burns in a cohort of firefighters were analyzed using NMR spectroscopy. We applied ^{1}H and 2D mononuclear ^{1}H-^{1}H COSY and TOCSY, ^{1}H-^{13}C HSQC and HMBC to identify EBC metabolites and analyze the chemical composition of the water-soluble fraction of fine particles. During this study, firefighters were exposed to particle mass ranging from 0.7 to 5.6 mg/m^3 with more than 50% composed of OC and EC (up to 2.5 mg/m^3), respectively, for an average period of 4 hours. In this paper we discuss the metabolic profiles of pre- and post-shift EBC samples obtained after targeted Partial Linear Square-Discriminant Analysis (PLS-DA) and cross-shift analyses of levels of EBC metabolites and relate them to smoke chemical composition and lung function. In particular, post-exposure increases in Formate (produced to fight inflammation) and Acetate (formed from the deacetylation of pro-inflammatory cytokines in the airway lining fluid) indicated acute lung inflammation after smoke inhalation.

408 Metabolic Phenotyping of Chloroacetanilide Herbicides in Earthworm Coelomic Fluid with 1H NMR and GC-MS

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Earthworms (*Eisenia fetida*) are vital members of the soil environment that are sensitive to contaminants, and monitoring their metabolism may be a useful indicator of soil health and ecotoxicity. Chloroacetanilide herbicides are a class of broad spectrum, pre-emergent herbicides that are among the most widely used herbicides worldwide. These compounds are persistent and mobile throughout the environment, especially once transformed to their ethanesulfonic and oxanilic acid analogs. Here, unique metabolic phenotypes of five chloroacetanilide herbicides and one enantiomer (acetochlor, alachlor, butachlor, racemic metolachlor, S-metolachlor, and propachlor) are observed in earthworm coelomic fluid using proton nuclear magnetic resonance spectroscopy (NMR) and gas chromatography-mass spectrometry (GC-MS). Univariate and multivariate statistical analyses were used to identify metabolic biomarkers and quantify perturbations in biochemical pathways that include carbohydrate metabolism, lipid biosynthesis, and amino acid metabolism. The score plots from multiblocked-orthogonal partial least squares-discriminant analysis (MB-OPLS-DA) showed good clustering among treatment groups, demonstrating that the metabolomic impacts of structurally similar compounds may be differentiated in the environment. Intriguingly, chirality affected the metabolic response to racemic metolachlor and S-metolachlor, with the results for each treatment on opposing sides of the score plots. These findings support the utility of coelomic fluid in monitoring the metabolic perturbations of chloroacetanilide herbicides on non-target organisms, and reveal specificity in the metabolic impacts of herbicide analogues in earthworms.

409 Gaining insight into AHR-mediated inhibition of osteogenesis through genomics and epigenomics

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Bone formation requires strict coordination of transcriptional regulatory pathways to direct commitment and differentiation of mesenchymal stem cells (MSCs) to mature osteoblasts. There is increasing concern, however, that exposure to environmental xenobiotic stressors may perturb the osteogenic pathways responsible for formation and maintenance of bone. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and other ligands to the aryl hydrocarbon receptor (AHR) are one such class of chemicals known to disrupt bone and cartilage formation in multiple in vivo models. In this study, we assess how embryonic 2,3,7,8-tetrachlorochlorodibenzo-p-dioxin (TCDD) exposure impacts axial osteogenesis in Japanese medaka (*Oryzias latipes*), a vertebrate model of human bone development. In vivo studies illustrate that exposure to TCDD results in attenuation of vertebral ossification and mineralization due to modulation in cell number and localization of transgene-labeled osteoblast and osteoblast progenitor cells. We also demonstrate AHR-mediated inhibition of osteogenesis in human bone-derived mesenchymal stem cells (hBMSCs) isolated from multiple donors. In vitro studies have enabled mechanistic insights into the role of TCDD repression on osteogenesis. Our data demonstrates differential expression of apical markers, key transcriptional regulators, and miRNA and lincRNAs known to play essential roles in osteogenic differentiation. Overall MSCs treated with TCDD exhibit a characteristic signature of repressed osteogenic differentiation in which cells retain mesenchymal-like properties. Current efforts seek to determine key epigenetic regulators that selectively promote lineage determination though modifications within the epigenome.

410 A New Test Solution to Improve Comparability of Non-targeted Analyses for Human Health and Toxicological Measurements

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With the introduction of high resolution mass spectrometry to liquid chromatography, the field of non-targeted ("omics"-style) analysis has rapidly expanded across many fields, including clinical and toxicological research. While non-targeted techniques are increasingly being developed by individual laboratories, the lack of apparent reproducibility and comparability of between-laboratory analytical results hinders their use for human health and public policy decision-making. This comparability issue can be the result of the lack of interlaboratory reproducibility of the instrumental technique and/or the inability to properly "align" the analytical data to provide a better comparison of interpreted results. At the National Institute of Standards and Technology, we are developing a multicomponent solution with the intent to address these challenges. The prototype solution contains many instrumental method-informative organic compounds, each with one (or more) characteristics that can be used to "describe" a whole LC-ESI-MS system. Instrumental parameters to be evaluated by this mixture include mobile phase conditions (e.g., pH, solvent purity, and gradient program); chromatographic column chemistry and quality; and ionization and fragmentation efficiency. An example of a set of test compounds is a homologous series of saturated fatty acid methyl esters, whose retention factor and methylene selectivity can be used to describe the hydrophobic retention of the entire chromatographic system. The goal of the test solution is to provide figures of merit that could fully describe an LC-ESI-MS method, which would then allow for the comparison of two different systems (or one system on two different days) and determination of the comparability, or lack thereof, of the results between these two systems. The solution could then be used to complement non-targeted results between laboratories, such as a interlaboratory metabolomics study, as supplemental information that would allow for a better-informed comparison of the results. Multiple test solutions were developed and evaluated in-house to determine the most informative, and broadly applicable, chemical compounds. After development, the test solution was applied to a wide variety of different LC-ESI-MS systems and instrumental settings to determine the variability of the test solution analysis. The development of the test solution mixture and the application of the mixture with different systems and settings will be presented.

411 Thyroid hormone disruptors identified in cat blood and house dust by Effect-Directed Analysis: Contribution to the exposome

M. Lamoree, L. Lucattini, N. Zwart, P. Cenijn, J. Koekkoek, P. Leonards, VU University Amsterdam / Environment and Health; J. Weiss, Stockholm University / ACES / Aquatic Sciences and Assessment; T. Hamers, VU University Amsterdam / Institute for Environmental Studies (IVM) / Chemistry

Indoor pet cats are exposed to thyroid hormone disrupting compounds (THDCs) through dust. We have investigated cat blood plasma as model for indoor human exposure to THDCs. Effect-Directed Analysis (EDA) combines biotesting, fractionation and chemical analysis to identify toxicants in complex matrices. EDA has already demonstrated to be a valid tool for identifying THDCs in blood serum [Simon et al., 2013] and environmental matrices [Ouyang et al., 2017]. For cat blood and house dust, extracts were prepared for EDA. The samples causing a response in the fluorescence T4-TTR binding assay were further fractionated into 96 well plates and tested in the bioassay again as well as screened for the presence of THDCs using liquid chromatography coupled to QuadrupoleTime-of-Flight Mass Spectrometry (LC-QToF-MS). The microfractionation was done using Ultra performance liquid chromatography (UPLC) into 96-well plates, thus avoiding time-consuming evaporation steps of the excess solvent and enabling high throughput. Prior to the fractionation of the dust and cat blood extracts, the procedure was optimized by the evaluation of the fractionation pattern of a test mixture of known thyroid hormone disrupting compounds, such as the OH-PCBs and several flameretardants. LC-QToF-MS using electrospray ionization (ESI) was used for suspect screening and nontarget analysis. The identification of suspects was done by sequential use of several instrument (Bruker Daltonics) software tools. For suspect screening, an 'in house' database was used containing around 11000 chemicals including chemicals reported in dust and consumer products (e.g. plasticizers and flame retardants), potential persistent and bioaccumulative (P&B) compounds, P&B transformation and by-products, impurities and pharmaceuticals. In addition, non-target screening was performed for the identification of the compounds responsible for the observed thyroid hormone disruptive effects.

412 Identifying compounds originating from consumer products in sewage sludge utilizing effects-directed, non-targeted LC-qTOF-MS/MS analysis

G. Black, University of California, Davis / Agricultural & Environmental Chemistry; T.M. Young, University of California, Davis; T. Anumol, Agilent

Endocrine disrupting compounds in the environment have been a topic of national and regional concern. The EPA Endocrine Disruptor Screening Program (EDSP) has identified hundreds of compounds that exhibit endocrine disrupting characteristics. To date however, there has been little research investigating endocrine activity of products formed during degradation of consumer product ingredients, such as occurs within wastewater treatment plants (WWTP), which are designed to achieve substantial reductions in concentrations of diverse organic compounds. Utilizing an effect-directed analysis (EDA) of endocrine active compounds in sewage sludge allows us to conduct a "bottom up" search for compounds that are not currently suspected as endocrine disrupting, and therefore not on the EDSP radar; or are transformation products originating from active ingredients found in consumer products. Twelve WWTPs throughout California have contributed sewage sludge samples for this research, which couples suspect and non-target High Resolution LC-MS/MS with cell-based CALUX (Chemically Activated LUciferase gene eXpression) bioassays capable of identifying estrogenic, androgenic and aryl hydrocarbon receptor interactions. This presentation will outline the method and workflow of this technique in addition to reporting a subset of universally present compounds in sewage sludge identified using target, suspect and non-target analytical approaches.

413 Non-targeted screening of DNA adducts as biomarkers for human exposure to PAHs in the environment with liquid chromatography tandem mass spectrometry

Y. Feng, C. Yao, Health Canada

Humans are constantly exposed to thousands of contaminants in the environment. Polycyclic aromatic hydrocarbons (PAHs) are a group of organic compounds containing two or more aromatic rings. They are released into the environment from both natural and anthropogenic sources such as combustion of organic substances and incomplete burning of coal, oil, gasoline, tobacco products and wood. PAHs are known to be bio-transformed by phase I metabolic enzymes to chemically reactive intermediates that may bind covalently to DNA to form DNA adducts that interfere with DNA synthesis and transcription, leading to DNA mutations and/or toxicity. Furthermore, binding of electrophilic PAH metabolites to DNA is thought to be a key step in the initiation of cancer. Therefore, measurement of those DNA adducts could be an indicator or biomarker of human exposure to PAHs in the environment and of the dose of the ultimate reactive metabolite. Rapid non-targeted approaches are desired to explore a broader scope of new biomarkers associated with the contaminants in the environment. Previous non-targeted analysis with retrospective analysis of the full scan data to identify DNA adducts is time consuming. In this presentation, we will report a non-targeted screening method for identification of covalent DNA adducts using a combination of neutral loss scan and product ion scan in a Q-trap system. The method was applied to non-targeted screening of DNA adducts in follicular cells from isolated ovarian follicles that were exposed to cigarette smoke condensate (CSC). Four DNA adducts, benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide-dG(BPDE-dG), phenanthrene 1,2-quinone-dG (PheQ-dG), B[a]P-7,8-quinone-dG (BPQ-dG) and 4-aminobiphenyl-dG, were identified in the follicular cells. The results also revealed that two oxidative biomarkers, 8-hydroxy-2-deoxy guanosine (8-OH-dG) and 8-isoprostane (8-IsoP), had strong correlations with the three DNA adducts, BPDE-dG, BPQ-dG and PheQ-dG, suggesting a strong link between the formation of covalent DNA adducts and DNA damaging oxidative stress. The method has also been successfully applied to investigate the selectivity of chemicals to modify the nitrogenous bases on DNA sequence. The results showed that each chemical had a different selectivity when it modified the DNA bases. The method has been demonstrated to be a potential tool to provide screening of unknown DNA adducts as biomarkers of human exposure to the parent contaminants in the environment.

414 Cross Validation Using Untargeted Metabolomics and Targeted Analysis in Children's Health Exposure Analysis Resources (CHEAR)

Y. Cui, NIEHS / Division of Extramural Research and Training; D.M. Balshaw, National Institute of Environmental Health Sciences, NIH

Untargeted metabolomics has been recognized as a useful tool in exposome research because of its capability to capture simultaneously hundreds of thousands chemical features that humans are exposed to, either from endogenous or exogenous sources, including small molecules that are potentially biomarkers of exposure, biological response, or phenotypic markers that are indicative of health outcomes. The current application of untargeted metabolomics in environmental health studies, however, has been limited by the incomplete coverage of environmental toxicants in the existing annotation databases, the difficulties in correlating exposure to biological pathways/health effects, as well as the lack of validation of untargeted discoveries themselves. The Children's Health Exposure Analysis Resources (CHEAR) funded by the National Institute of Environmental Health Sciences (NIEHS) is established to expand the study of environmental exposures that could potentially impact children's health. CHEAR has extensive capabilities in both untargeted metabolomics and traditional biomonitoring (targeted analysis), the latter of which is considered the golden standard for assessing exposure to a wide range of environmental factors in molecular epidemiology studies. This talk will focus on the targeted and untargeted analysis capabilities of CHEAR and how the two approaches can be coupled in children's environmental health studies for simultaneous analysis of multiple environmental chemicals and validation of previously unknown exposures that may impact children's health outcome.

Alternative Approaches to Animal Testing for Ecotoxicity Assessments

415 A brief history of nearly everything alternative

A. Lillicrap, NIVA / Ecotoxicology and Risk Assessment

Over the last two decades there have been significant progress towards developing alternative approaches for environmental hazard and risk assessment purposes. Some of these developments have been driven by legislative requirements, to avoid unnecessary use of vertebrate organisms for aquatic ecotoxicity assessments. Other drivers include reducing cost or improving the data that are generated. The issue with ensuring that any alternative approach becomes accepted, is that the methods need to be validated to show how reproducible the method is, it also needs to be relevant for the purpose of the test and most importantly needs to be accepted by regulatory authorities. These additional principles are being referred to, collectively with the traditional 3Rs, as the 6Rs for alternative approaches. One notable alternative approach for ecotoxicity testing is the fish embryo toxicity (FET) test that was developed over 20 years ago as a potential alternative approach to the fish acute toxicity test. However, the development and acceptance of this approach has not been straightforward. This presentation gives a historic perspective on the development, validation and possible future implementation of the FET test for ecotoxicity assessments. In addition, an overview of a recent Environmental Toxicology and Chemistry Focus Article, produced by steering committee members of the SETAC Global Animal Alternatives Interest Group, will be presented.

416 Age Matters: Developmental Stage of Zebrafish (Danio rerio) Influences Bioconcentration, and Survival and Behavioral Photomotor Response Thresholds

L.A. Kristofco, S. Haddad, Baylor University / Environmental Science; K. Chambliss, Baylor University / Chemistry; B.W. Brooks, Baylor University / Environmental Science

Toxicology information for the tens of thousands of compounds in commerce is severely lacking, and with continual introduction of new substances, this global chemical space is increasing in complexity. Significant hurdles exist for the production of new toxicology data because traditional whole organism studies are costly, time intensive, and low throughput. To meet these goals alternative methods have been developed that shift the focus from a traditional study design to a higher throughput based design. However, as these efforts are advanced, evaluations of the newly introduced methodologies are warranted. Here, we examined the Fish Embryo Toxicity Test's (FET; OECD 236) opportunities and limitations with the zebrafish, *Danio rerio*, and a model antihistamine, diphenhydramine (DPH). 72 hour DPH studies were conducted during embryonic, elutheroembryo, and larval stages, and subsequent lethality and photomotor responses were recorded. For both traditional and alternative behavioral measurements larval stages were most sensitive, with significant behavioral perturbations observed at environmentally relevant levels (200 ng/L DPH). To further understand this age dependent toxicity relationship, DPH uptake was evaluated for chorionated and dechorionated embryos, and in early and late larval periods. Interestingly, age had a much greater influence on uptake than did the chorion. For both chorionated and dechorionated embryos steady state was rapidly reached and persisted through the natural hatching period (48 hpf). Following this period, a sharp increase in DPH body burden was observed. Conversely, during early and late larval periods DPH uptake resembled that previously reported in adult fish. This plausibly links physiological events such as gill development to increased body burden and thus toxicity. Future studies are needed to determine whether such age specific increases in uptake and toxicity extend to other fish developmental models and organic contaminants.

417 Field validation of ultrasound as a non-lethal tool to measure sentinel fish liver size for environmental effects monitoring

A. Manek, University of Saskatchewan / Veterinary Biomedical Sciences; V. Palace, IISD-Experimental Lakes Area; P. Borrett, University of Saskatchewan; L. Hrenchuk, IISD-Experimental Lakes Area; M. Murdoch, Stantec Consulting Inc; L.P. Weber, University of Saskatchewan / Veterinary Biomedical Sciences

The environmental effects monitoring (EEM) program evaluates potential impacts of anthropogenic activities (e.g. release of metal mining and pulp mill effluents) on aquatic receiving environments. EEM regulations recommend lethal sampling of 20 male and 20 female fish of 2 different species to study body condition, liver size (hepatosomatic index-HSI), and gonad size (gonadosomatic index-GSI). Repeated lethal EEM sampling in aquatic ecosystems with populations of fish that are endangered or have low productivity can have negative population impacts. The importance of alternative, non-lethal methods for EEM programs has been gaining precedence. Ultrasound is a non-invasive tool that can be used to study HSI or GSI in fish. However, it's applications for environmental monitoring have been limited. Our initial laboratory studies provide strong empirical evidence for the accuracy and sensitivity of using ultrasound as a non-lethal method versus traditional lethal gravimetric methods to measure HSI in rainbow trout (*Oncorhynchus mykiss*). We extended the ultrasound technique validation to the field in different seasons in collaboration with scientists from IISD Experimental Lakes Area (IISD-ELA) in sentinel fish species with compact liver structure such as lake trout (*Salvelinus namaycush*) and northern pike (*Esox lucius*) compared to species with a diffuse liver such as white sucker (*Catostomus commersonii*). We used whole body slices of white sucker obtained by lethal sampling to generate 3-dimenstional (3D) constructs of the diffuse liver. We correlated the HSI generated from the slice 3D construct with the non-lethal ultrasound method. In addition, we compared the HSI of lake trout generated by traditional lethal gravimetric method versus non-lethal ultrasound method. Our preliminary findings suggest that seasonality, liver structure, and reproductive status of fish influence our ability to visualize the liver using the ultrasound. Despite this variation, there was good correlation between HSI generated by different lethal methods versus non-lethal ultrasound in wild white sucker and lake trout. Multiple field seasons will further test the ultrasound technique in these sentinel species to further examine the utility of using ultrasound as an alternative for non-lethal EEM programs to monitor HSI.

418 Assessing chemical exposure and ecological impacts of environmental surface waters using cell culture-based metabolomics

Q. Teng, USEPA / EMMD / National Exposure Research Laboratory; W. Wang, H. Zhen, D. Ekman, USEPA / National Exposure Research Laboratory; D.L. Villeneuve, USEPA / National Health and Environmental Effects Research Laboratory; J.E. Cavallin, USEPA / Mid-Continent Ecology Division; G.T. Ankley, USEPA / National Health and Environmental Effects Research Laboratory; P. Bradley, USGS / SAWSC; T. Collette, USEPA / National Exposure Research Laboratory

Waste water treatment plants (WWTPs), as well as industrial and agricultural operations release complex mixtures of anthropogenic chemicals that negatively affect surface water quality. Previous studies have shown that exposure to such complex chemical mixtures can produce adverse health effects in resident organisms. Traditional methods using live animals (e.g., fish) for monitoring and assessing contaminant exposure and impacts in affected ecosystems are both resource and time intensive and thus often impractical for screening large numbers of impacted sites. Cell culture-based metabolomics has the ability to rapidly assess environmental impacts produced by such exposures and to investigate the components of contaminant mixtures that are likely responsible for those impacts. In this study, we applied cell-based metabolomics to detect biological impacts of environmental surface waters collected from 38 stream sites across the U.S. Zebrafish liver cells (ZFL) and two human cell lines (HepG2 and LN229) were exposed to media prepared with surface water samples for 48h. Intracellular, polar metabolites of all three cell lines were detected using nuclear magnetic resonance (NMR) spectroscopy and high-resolution liquid chromatography mass spectrometry (HR LC-MS). The biochemical pathways most impacted by the exposures were determined based on relative changes in the levels of metabolites across the sites. Here we report the methodology used to identify these pathways as well as a comparison of responses across all 38 streams.

419 Validation of a Glucocorticoid Receptor Effects-Based Environmental Sample Screening Tool

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Glucocorticoid activity has been detected, using in vitro effects-based monitoring tools (e.g., transcriptional activation bioassays), in waste and surface waters domestically and around the world. A review of the existing literature confirms that many different glucocorticoid receptor-specific pharmaceuticals have been detected in water samples. Therefore, we have further validated the sensitivity of the glucocorticoid receptor transcriptional activation bioassay using 10 known GR agonists as well as one antagonist. CV1a cells, devoid of endogenous GR and androgen receptor, were adenovirally transduced with the human GR and MMTV-luciferase genes. Cells were then treated with individual GR agonist or dexamethasone + antagonist (mifepristone) to determine individual compound potency and efficacy. Efficacy (%maximum response) varied between compounds and potency, noted by nominal EC_{50} values, ranged over several orders of magnitude; triamcinolone acetonide ($8.914x10^{-11}$M) < fluticasone propionate ($8.829x10^{-11}$M) < flucinonide ($1.278x10^{-10}$M) < dexamethasone ($2.118x10^{-10}$M) < betamethasone (2.175 $x10^{-10}$M) < fludrocortisone ($4.932x10^{-10}$M) < corticosterone ($1.083x10^{-8}$M) < prednisolone ($3.873x10^{-8}$m) < 21-hydroxyprogesterone ($8.097x10^{-8}$M) < cortisone. Further, a GR antagonist standard curve, suited to evaluate GR antagonistic activity (MifEqs) in environmental mixture samples, was optimized by co-exposing cells to dexamethasone with a range of mifepristone concentrations. The IC_{50} of mifepristone, in the presence of 1pM dexamethasone, was $8.355x10^{-10}$. Validating this screening tool using commonly detected GR-specific pharmaceuticals will allow the determination of individual contributions of detected GR agonists and antagonists in future surface and waste water samples. Abstract does not necessarily reflect USEPA views or policy.

420 Screening the ToxCast Phase I, II, and e1K Chemical Libraries for Inhibition of Deiodinase Type 1,2, and 3 Enzyme Activity

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Thyroid hormone (TH) signaling and homeostasis is dependent upon coordination of multiple key events including thyroidal iodide uptake and hormone synthesis, and peripheral metabolism and elimination. Deiodinase enzymes play an essential role in converting the pro-hormone thyroxine to the active hormone triiodothyronine. They also convert these THs to inactive forms via further removal of iodide from the substrate hormones. The activity of the deiodinases has been recognized as an important endpoint to include in the development of screening assays to identify thyroid hormone disrupting chemicals. To address the lack of data regarding the potential for chemicals to inhibit these enzymes a research effort was initially focused on human deiodinase type 1 (D1). We utilized an adenovirus expression system for production of D1 enzyme, established robust assay parameters for non-radioactive determination of iodide release by the Sandell-Kolthoff method, and employed a 96-well plate format for screening chemicals. An initial set of 19 chemicals was used to establish the performance of the assays. This successful approach was extended to include human deiodinase types 2 and 3. Over 1800 unique chemicals, primarily from the USEPA's ToxCast phase 1_v2, phase 2, and e1K chemical libraries, were tested in each of the three screening assays. Chemicals were initially screened at a single high concentration of 200 µM to identify potential enzyme inhibitors. The majority of the chemicals did not inhibit deiodinase activity in this initial screen. Across the three enzymes, only 15-20% of the chemicals produced enzyme inhibition of 20% or greater. Approximately half of these inhibited the enzymes by greater than 50% compared to control enzyme activity. Chemicals that inhibited deiodinase activity by greater than 50% were further tested in concentration-response mode to determine relative potency. This work presents an initial effort toward screening chemicals with the potential for affecting thyroid hormone status via inhibition of deiodinase activity. It sets the groundwork for development and evaluation of structure-activity relationships for deiodinase inhibition, and provides a dataset for the targeted selection of chemicals for testing to identify deiodinase inhibition adverse outcomes. This abstract does not necessarily reflect USEPA policy.

421 Deriving Predicted No-Effect Concentrations in Diverse Geographies for Use in Eco-TTC Estimations

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Ecological Thresholds for Toxicologic Concern (eco-TTC) employs an assessment of distributions of Predicted No-Effect Concentrations (PNECs) for compounds following chemical grouping. Grouping can be by mode of action, structural fragments, or by chemical functional use. Thus, eco-TTCs summarize the wealth of ecotoxicological information as probability distributions of PNECs and the 5th percentile lower value is chosen to represent the eco-TTC per se. Ecotoxicological hazards for untested chemicals, grouped using the same attributes, could be conservatively estimated. PNEC determinations vary fundamentally by regulatory jurisdiction. Application factors assigned to different levels of ecotoxicological data (species breadth, acute or chronic toxicity) result in different extrapolations for a potential “safe concentration” of a chemical. We compared PNECs derived by US and European environmental regulatory PNEC approaches in detail for ~5000 compounds and Japan and Canadian environmental PNEC approaches for a subset of these. Algorithms were written in R for US and Europe PNEC processes, then implemented into an eco-TTC web application as envisioned in Belanger et al. (2015). Cumulative PNEC probability distributions for European, Canadian, and Japan approaches are somewhat more conservative than the US approach driven principally by smaller assessment factors applied to data sets at earlier stages of hazard assessment. For example, the AF for the US when a full toxicity data set is available for all 3 trophic levels and a chronic test is available on the most sensitive acute species is 10; however, in other jurisdictions this may be as high as 100. On average, European PNECs were 11 times more conservative than US PNECs. PNEC distributions across geographies are driven by the large number of compounds that lack full chronic toxicity data. All assessments derive similar PNECs when full chronic toxicity data sets are available but this is only ~5% of cases encountered. The PNEC derivation logic, embedded in the eco-TTC web application, will be a useful tool to allow assessors to quickly and consistently compare hazard extrapolations across geographies minimizing animal testing requirements and maximizing use of existing information. The views, conclusions and recommendations expressed in this article are those of the author and do not necessarily represent views or policies of the European Commission or the USEPA.

422 Ecological Threshold for Toxicological Concern (eco-TTC) - Applications for Environmental Risk Assessment in Various Contexts

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The Threshold for Toxicological Concern (TTC) is well-established for assessing human safety but has only recently been explored in the ecological context. Ecological TTCs summarize the wealth of ecotoxicological information as Predicted No-Observed Effect Concentrations (PNECs) on diverse chemical substances in the form of probability distributions. These enable the prediction of untested chemicals based on a structural attribute, mode of action, or functional use. The approach may be useful for assessing chemicals at early tiers of the risk assessment process, providing hazard perspective on chemicals that lack QSARs, guiding product development discussions, and assisting read across or category justifications. An ecotoxicological database was developed based on recent assessments of published data and international chemical management programs. This ecotoxicity data is associated with physical chemistry data and curated taxonomic information for the organisms tested, including a process to conclude acute and chronic effects as well as identify the PNEC for exposed ecosystems based on depth and breadth of data. Several mode of action schemes are also included to facilitate development of a best approach for grouping compounds. To make these data accessible and useful to stakeholders, the dataset was transitioned from Microsoft Excel and Access into a modern MySQL format, allowing for a for format that is relational and scalable, facilitating easy access, sharing, and integration with other datasets and tools. The dataset is accessed via a web-based query system that is integrated with PNEC calculator and probability distribution tools. The novel interface allows users to explore the data, upload additional datasets, derive threshold values based on specific criteria, and explore the potential use and application of the ecoTTC concept. An international workshop was held to discuss and evaluate the feasibility of the eco-TTC approach, which included evaluation of several case-studies based on particular decision-contexts (e.g., prioritization and screening, chemical risk assessment, site specific risk assessment, mixtures, product development, criteria development). This presentation will highlight the discussions and conclusions from that workshop, including exploration of how this approach could be applied and integrated into evaluation strategies (e.g., IATA).

Systems Biology for Ecotoxicology – From Gene to Ecosystem – Part 1

423 Genome resources and their use in identifying deleterious effects in ecotoxicological studies of true frogs

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Ranids are the largest family of frogs in the world. Common examples include the wood frog, Northern leopard frog, and the North American bullfrog. Amphibians are regarded as sensitive sentinels of the environment. However there is a paucity of genomics resources available for the application to determining bioindicators linked to adverse outcome pathways. We have developed several new genomics tools including the first true frog genome from the North American bullfrog, de novo assembly transcriptome pipelines, and RNA-seq analysis pipelines that we have applied to identify potential thyroid hormone (TH) endocrine disruption in bullfrog tadpoles. We examine the responsiveness of multiple tissues to the thyroid hormones, thyroxine and 3,3',5-triiodothyronine, and relate these responses to disruptions in development, behavior, and chemosensation. Novel bioindicators were then applied to evaluate the efficacy of removing TH-disrupting activity in municipal wastewater treated with secondary treatment. These studies demonstrate the utility of systems-level analysis for the linkage of molecular bioindicators to adverse outcomes. Supported by NSERC Canada, Compute Canada, and Genome British Columbia.

424 Effects of polycylic aromatic hydrocarbons and hypoxia on Fundulus grandis transcriptomics

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Although the toxicity of polycyclic aromatic hydrocarbons (PAHs) in fishes has been well-studied, the mechanisms by which PAHs exert their toxicity are not well understood. Our previous work, showed that the toxicity of PAHs to fish larvae was enhanced under hypoxia. Therefore, the objective of this work was to use transcriptomics to unravel the molecular changes occurring after co-exposure to both stressors. *Fundulus grandis* larvae (< 24 h post hatch) were exposed to varying oxygen levels (dissolved oxygen 2, 6 ppm) combined with a low concentration of high energy water accommodated fractions (HEWAF) (∑PAHs 15 ppb) for a total of either 24 h (hypoxia) or 48 h (normoxia). Transcriptional changes were quantified using RNA-Seq. There were more differentially expressed genes induced by PAH exposure (380) than hypoxia (110). For PAHs, gene expression changes were related to cardiac (e.g., *fgf7* and *mb*) and hepatic (e.g., *ahrr, por*) function and disease. Interestingly, a different set of genes associated with cardiac (e.g., *fbxo32*) and liver (e.g., *ucp2, upp2*) pathology were also significantly affected under hypoxia. The phase II detoxifying enzyme *ugt1a1* was significantly induced across all PAH exposure comparisons and was identified as a potential biomarker. Although there was no induction of *cyp1a* to PAH exposure, other aryl-hydrocarbon receptor (AhR) pathway-associated genes changed in expression (*ahrr, por*). This work reaffirms the need for more studies on the transcriptional changes induced by pollutants and environmental stressors, such as hypoxia, in early life-stage fishes.

425 The pesticide fipronil affects transcriptional networks related to mitochondrial dysfunction and methylation in zebrafish embryos (Danio rerio)

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The phenylpyrazole fipronil is a widely used insecticide designed to inhibit γ -amino-butyric acid (GABA) receptors, the major inhibitory neurotransmitter in the central nervous system. Fipronil has been detected in some water systems in the ng/L range, and is reported to be neurotoxic. To address the risks associated with fipronil exposure, we measured morphological, physiological, and molecular responses in zebrafish (*Danio rerio*) embryos following a 48 hour exposure (20 ng/L – 2 mg/L). Survival was not different than controls following treatments below 200 µg fipronil/L but was ~20% higher with concentrations above 200 µg fipronil/L. Once the embryos hatched, they underwent a 7 day depuration phase. At 9 days post-fertilization (9 dpf), body length and notochord length were not different than controls for any dose. To assess sub-lethal effects, transcriptome profiling was conducted in 9 dpf larvae following 48 hour exposure + 7 dpf depuration to environmentally relevant concentrations of fipronil (200 ng fipronil/L), as well as two higher concentrations of the pesticide (200 µg fipronil/L and 2 mg fipronil/L). Transcriptome profiling revealed that all three concentrations affected pathways related to chromosome condensation and the metabolism of estrogens and androgens as well as genes related to methylation. In addition, 200 ng fipronil/L down-regulated genes related to the circadian clock, histone and DNA methylation, and histone acetylation, while the highest dose increased networks related to immune function (e.g. lectin-induced complement pathway and the alternative complement pathway). The two highest concentrations of fipronil increased the expression of transcriptional networks associated with mitochondrial respiratory chain dysfunction and mitochondrial protein transport. As such, we exposed 24 hpf embryos to fipronil for 24 hours and measured oxygen consumption rate to assess mitochondrial function. There were no differences in basal and maximal respiration in the embryos nor ATP production, and fipronil did not affect mitochondrial bioenergetics. This study suggests that fipronil at environmentally relevant concentrations does not adversely affect the survival or morphology of fish embryos, however sub-lethal endpoints should be examined to more fully characterize the long term effects of fipronil exposure in larval fish.

426 Metabolomic Investigations of the Temporal Effects of Exposure to Pharmaceuticals and Personal Care Products and Their Mixture in the Eastern Oyster

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The eastern oyster (*Crassostrea virginica*) supports a large aquaculture industry and is a keystone species along the Atlantic seaboard. In the environment, oysters are routinely exposed to a complex mixture of contaminants that increasingly includes pharmaceuticals and personal care products. Unfortunately, the biological effects of chemical mixtures on oysters are poorly understood. We utilized untargeted GC-MS metabolomics to quantify the response of the oysters exposed to fluoxetine, N,N-diethyl-meta-toluamide (DEET), 17α-ethinylestradiol (EE2), diphenhydramine and their mixture. Oysters were exposed to 1 µg/L of each chemical for ten days, followed by an eight-day depuration period. Adductor muscle (n = 14/treatment) was sampled at days 0, 1, 5, 10 and 18. Changes in the relative abundance of individual metabolites in each treatment were used to identify potential biomarkers of exposure to these contaminants. Detected metabolites were associated with cellular energetics (citric acid and glyoxylate cycles and fatty acid and sugar metabolism), as well as amino acid metabolism and the urea cycle. Principle component analysis (PCA) was used to discern the relationships between individual treatments and their mixture. Following 24-hr of exposure, diphenhydramine had the greatest effect on the metabolome (as evidenced by PCA class separation) with EE2 exposed oysters being closest to control. From days 5-10, diphenhydramine, EE2 and DEET appear to cluster apart from fluoxetine and their mixture, potentially indicating similar biochemical responses within these clusters. Following depuration, the metabolomic profile of fluoxetine and EE2 were no longer statistically different from controls while the other treatments still appear to elicit effects on the oyster metabolome. Interestingly, the mixture did not appear to directly cluster with individual treatments suggesting its physiological effects are unique when constructing PCA models across day of exposure. This research highlights the utility of untargeted metabolomics in developing exposure biomarkers for compounds with differing modes of action, while exposing several challenges in interpreting single chemical versus mixture responses in bivalves.

427 Integration of chemical, organismal, and transcriptomic data reveals landscape-specific exposure effects to complex chemical mixtures

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Aquatic habitats are often contaminated with complex mixtures of pollutants. Interpreting biological effects caused by mixture exposures is an ongoing challenge in ecotoxicology and is expected to improve with chemical and geographical data. Here, we employed integrated chemical and biological analyses to determine how environmental mixtures affected biological responses in watersheds with different landuse. A better understanding of the relationships between landuse, contaminant occurrence, and exposure effects will increase predictive power for regulators and managers. Adult male fathead minnows (*Pimephales promelas*) were exposed to water from different locations within the Shenandoah River watershed (VA, USA) in 2014 and 2015. The exposure locations were chosen to capture unique landuse in surrounding watersheds, including agricultural, municipal, mixed-use, and forested sites. Endpoints from multiple levels of biological organization were measured, including condition factor, gonadosomatic index (GSI), number of nuptial tubercles, and hepatic gene expression profiles. Water samples were taken 4 times during the fish exposure and analyzed for over 460 chemical constituents. Each location had a unique chemical profile that was generally consistent with landuse. Whole-organism and molecular responses also differed between the locations. In 2014, fish exposed at agricultural and WWTP impacted sites showed signs of endocrine disruption including a reduced number of nuptial tubercles and decreased GSI. However, transcript biomarkers of estrogen exposure, including *er1, er2, vtg1,* and *vtg3* showed little to no differential expression, suggesting that these fish were not affected in a significant way by estrogenic compounds in the mixture. Pathway analysis of transcriptomic data revealed that lipid related processes were affected by exposure to agricultural, WWTP impacted and mixed-use watersheds. Immune-related processes were down-regulated at the agricultural site and up-regulated at the mixed-use site. Hierarchical clustering of total transcriptome profiles showed individuals generally clustered by exposure location, demonstrating that exposure to water from sites with different landuse results in unique and site specific responses at the transcript level. These data generate new hypotheses regarding the effects of exposure to different types of complex mixtures and demonstrate the value of our complex mixture/landscape research approach.

428 AOPXplorer: From networks to adverse outcomes

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Risk managers and risk assessors will soon be flooded with molecular and phenotypic data coming from omics and high throughput screening technologies. Although the Adverse Outcome Pathway framework provides a means to begin to anchor, interpret, and understand these data, biology does not occur in single pathways – it is networks of pathways interacting with each other that ultimately lead to the complexity of adverse outcomes. Currently there is a dearth of tools available to integrate and make sense of all of this systems biology data. We built the AOPXplorer to address this need, allowing us to integrate, explore, and visualize the data in ways that will help us understand what the molecular responses mean within a larger biological context, and to facilitate the automated screening of hazards and perform dose-response assessments in a more rapid fashion. The AOPXplorer is a cytoscape tool that models and integrates the data, allowing the computer to quickly perform hazard identification on chemical data by comparing activated key events against the set key events that are sufficient to infer that an adverse outcome is likely to occur. This allows users to quickly scan through the list of potential adverse outcomes, and see visually how the computer made these inferences by overlaying the molecular data onto adverse outcome pathways. AOPXplorer is therefore a great tool to visualize AOP networks and help build testable hypotheses. The AOPXplorer will also facilitate the creation of analysis pipelines, such as automated point of departure calculations.

429 Development of Metabolic Models Predictive of Intersex in Teleost Fish

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Intersex has been increasingly reported in fish residing in rivers and impoundments across North America and around the world. The development of testicular oocytes (TO, the most common intersex phenotype) among male fish is often linked to endocrine disruption via exposure to environmental estrogens. Reduced sperm fertility and motility reported in intersex fish has raised concerns about potential population level effects, yet little is understood about associated biochemical changes. Existing methods to positively identify TO are lethal, and estimation of prevalence among wild populations requires sacrifice of large numbers of adult males. Advances in the field of metabolomics allow for the simultaneous examination of hundreds of endogenous metabolites in body tissues, including some that can be collected non-lethally (e.g. blood, skin mucus). Identification of unique metabolic profiles in intersex fish may aid in identification of key cellular pathways and lead to development of biomarkers. We investigated metabolic profiles of macroscopic male largemouth bass with TO counts ranging from 0 – 164 (per longitudinal histological section) from an impoundment in the Piedmont region of Georgia. The levels of several metabolites from the liver, gonad, mucus, and blood plasma were significantly ($p< 0.05$) correlated with the number of oocytes detected in the testis, lending insights to the mechanisms of intersex induction in these fish, and allowing for the development predictive models. Development of methods to detect intersex biomarkers, particularly in non-lethal tissues/fluids would greatly enhance our ability to sample larger numbers of wild fish and allow for repeated sampling of individuals. In addition, elucidation of associated pathways could inform adverse outcome pathways and ecological risk assessments by increasing our understanding of biochemical mechanisms, spatial and temporal trends, causative factors and adverse effects associated with intersex on an individual and population level.

430 Disruption of the stress response in teleosts: The complexity and challenges of interrenal gland transcriptomics following fluoxetine exposure

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In mammals, the adrenal gland is separated, positioned on the kidneys in mammals, and is composed of the multilayered steroidogenic cortex where glucocorticoids are produced. The medulla is the site of chromaffin cells and thus, catecholamine release. In teleostst, the interrenal in the head kidney is the site of steroid synthesis, and chromaffin cells are found interspersed within the tissue. The hadrenocortical complex is not separated from the kidney, so it can be considered an interrenal-kidney complex (IKC). Dissection of steroidogenic tissue is not easy and tissue-level effect or gene expression analysis will invariably reflect a complex mixture of cell types. We have approached this challenge from the perspective of whole tissue transcriptomics to characterize the endocrine disruption in the zebrafish "interrenal" following a 6 day early-life exposure to the antidepressant fluoxetine (FLX), which is released to the environment via sewage. FLX decreases basal, stress- and ACTH-induced whole body cortisol production in males only. Adult (6 months) male IKCs were dissected and subjected to RNA-seq and this included control fish and those exposed to fluoxetine (0.54 and 54 micrograms/L). A total of ~17,000 transcripts were identified. Gene set enrichment analysis and sub-network enrichment analysis revealed that early FLX exposure significantly modified cholesterol biosynthesis and transport, steroidogenesis and beta-adrenergic signaling, indicating disruption of adrenocortical and adrenomedullary functions. Importantly, there were also numerous (>400) known pathways and cell processes affected by FLX treatment. These include Vasopressin Receptor Signaling (kidney function), T-cell Receptor Signaling (immunology, cell survival), among many. Our data indicate that whole tissue transcriptional profiling and enrichment analysis correspond to the phenotype (reduced cortisol) of interest. The multitude of other effects remain challenging to interpret. These other pathways could be reflective of direct effects of FLX, or they could be downstream, and may result from the chronic cortisol suppression observed. Regardless, our data indicate that early, waterborne exposure to FLX has persistent disruptive consequences for the IKC in adult male zebrafish. Acknowledgements: FRQNT scholarship (MVC), and grants from NSERC (TWM, VLT), uOttawa (TWM), College of Veterinary Medicine (CJM), University Research Chair Program (VLT), NASCE (MVC) and Health Canada (CLY).

Epigenetic and Evolutionary Effects of Pollutants – New Challenges for Long-term Ecological Risk Assessment

431 Genome-wide Analysis of Cadmium Induced Mutation in the Context of Nucleosome Composition

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Understanding how epigenomic patterns and oxidative stress contribute to patterns of genome-wide mutation is needed to understand the origins of complex disease and the variation driving all evolutionary processes. To determine how chronic levels of oxidative stress influence the rate and spectrum of germ line mutation in the context of epigenomic background, we completed a long-term mutation accumulation (MA) experiment utilizing the microcrustacean *Daphnia pulex*. In an MA experiment, initially genetically-identical lines are propagated through repeated bottlenecking, independently, thereby reducing the effective population size to 1, and thereby preserving all mutations except for those that cause immediate lethality and/or sterility. We propagated, 1) twelve MA lines in standard laboratory culturing, and 2) twelve lines in the presence of chronic levels of cadmium (Cd) for more than 2000 generations. Relative to lines in standard laboratory conditions, lines propagated in Cd had significantly higher rates of specific mutation classes (specifically, A/T->G/C). Furthermore, while lines in standard laboratory conditions exhibited a random distribution of mutations relative to nucleosome position, lines in the presence of cadmium were significantly enriched for mutation at genomic regions of high nucleosome occupancy. We also report a spontaneous mutator phenotype that arose in the course of the experiment. Interestingly, this mutator phenotype showed an opposite trend, one in which mutations are highly enriched in nucleosome-depleted regions. This study gives insight to the role long term, chronic levels of oxidative stress contributes to the spectrum of genomic mutation and gives insight into its role in disease occurrence, progression and susceptibility.

432 Repeated and widespread adaptive evolution of pyrethroid resistance among pyrethroid-exposed wild Hyalella azteca

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Pyrethroid pesticides are used in residential, landscaping, and agricultural settings and are frequently detected in both sediment and water at levels toxic to the epibenthic amphipod and ecotoxicological model *Hyalella azteca*. As an apparent adaptive response to this exposure, up to 550-fold pyrethroid pesticide resistance has been observed among several wild populations and is correlated with the presence of one of two non-synonymous base pair substitutions at the pyrethroid target site, the voltage gated sodium channel. These substitutions, methionine-to-leucine (M918L) and leucine-to-isoleucine (L915I), were first identified among pest insects exposed to pyrethroids. By surveying a total of 16 wild populations of *H. azteca* throughout California fromboth undeveloped (no surrounding pyrethroid use) and developed (expected pyrethroids from urban or agricultural use) regions, the present study confirms the direct relationship between pyrethroid exposure and evolution of adaptive pesticide resistance conferred via resistance alleles in *H. azteca* populations. In this study, *H. azteca* from undeveloped regions exhibited sensitivity to cyfluthrin, a pyrethroid, in the same range as that of sensitive laboratory populations of *H. azteca*. In contrast, populations from developed sites displayed resistance to cyfluthrin ranging from 10- to 420-fold higher than non-resistant populations. Resistance alleles were documented in all populations with resistance phenotypes and appeared in three different species groups within this complex, indicating repeated independent selection. The most commonly detected mutation was the L925I, which occurred at high frequencies (greater than 80%) in resistant populations collected from developed regions. Further, five *H. azteca* populations from regions of primarily urban land use were fixed for the L925I resistance allele, indicating strong selection from residential and landscaping pyrethroid use. The repeated selection for resistance alleles in *H. azteca* indicates these populations are insufficiently protected by current pyrethroid regulations, and this work has wide-ranging implications, from the interpretation of biological assessments to the evolutionary fitness of resistant populations of *H. azteca* in the face of this and other anthropogenic pollution.

433 Evolution in response to pollution in Gulf killifish (Fundulus grandis) from Galveston Bay, Texas, USA

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Anthropogenic contamination associated with industrial activity is a widespread and active threat to the stability of organisms. The Houston Ship Channel (HSC) is one example of a heavily impacted environment, where industrial activity has contributed to extreme levels of pollution with various classes of contaminants, such as polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs), polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). This dissertation studies the impacts of chronic multi-generational exposure to industrial contamination on the population structure, resistance and demography in a keystone coastal species – Gulf killifish (*Fundulus grandis*). We have characterized their sensitivity to contaminants in populations from 12 locations across Galveston Bay, as well as the contamination levels at those sites. We found a gradient of resistance that was positively correlated with contaminant concentrations. This resistance was also correlated to a suppression of the aryl hydrocarbon receptor pathway (AHR), as estimated via the activity of a down-stream regulated enzyme – cytochrome P450 1A (CYP1A). To better understand the impacts of this adaptation, we evaluated the cross-resistance and fitness cost of populations to mechanistically and environmentally relevant stressors, but we were unable to confirm any fitness costs. We showed that the heritability of this resistance is biparental and multi-generational, which suggest that this is a genetic trait. Finally, we performed a full genome resequencing of seven populations along this gradient of resistance and discovered that genomic regions under selection in adapted populations included the AHR pathway. Here we show the specific genetic changes that have allowed Gulf killifish to adapt to anthropogenic contamination in the HSC.

434 The effects of oil and hypoxia exposure on establishment of DNA methylation patterns in developing sheepshead minnows (Cyprinodon variegatus)

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The 2010 Deepwater Horizon oil spill resulted in substantial oiling to the northern Gulf of Mexico (nGOM) coast and exposed many estuarine fish to oil contamination. The arrival of oil slicks along the nGOM shoreline during spring and summer months coincided with fish spawning and periods of environmental stress, such as hypoxia. Both oil and hypoxia induce significant changes to gene expression in developing fish, however, there is little information regarding their effects on epigenetic modifications, such as DNA methylation, which can permanently alter gene expression. To examine these effects, we exposed sheepshead minnows (*Cyprinodon variegatus*) at three developmental stages to oil, hypoxia, or both for 48 hours. Using qPCR, we measured changes in mRNA expression of DNA methylation enzymes (DNMT1, DNMT3a, and DNMT3b) immediately after exposure and after 48 hours of depuration. Additionally, we analyzed transcriptomic changes using RNA sequencing, and measured total genomic methylation (after depuration) using a colorimetric assay. Our preliminary results indicate that oil exposure significantly down-regulates DNMT mRNA expression under normoxic conditions, however, under hypoxic conditions there is no significant change in DNMT expression. These data represent the first report in fish of oil-induced transcriptional changes to DNMT enzymes and elucidate the combined effects of oil and hypoxia on DNMT expression. Additionally, this study addresses whether DNMT transcriptional changes result in altered global DNA methylation patterns, which may enhance our understanding of whether oil–induced changes in gene expression might be permanent due to the alteration of epigenetic profiles.

435 Regulation of microRNAs in mahi-mahi (Coryphaena hippurus) exposed to Deepwater Horizon oil

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The Deepwater Horizon (DWH) oil can cause cardiotoxicity in a number of fish species, but the molecular mechanisms are not well understood. One possible regulatory mechanism is by disrupting normal expression of microRNAs (miRNA) that play key roles in diverse biological processes including heart development. In our study we aimed to evaluate the effects of DHW oil on miRNA expression. Mahi-mahi (*Coryphaena hippurus*) embryos were exposed to WAF from weathered slick oil and non-weathered source oil to compare the expression patterns of miRNAs with actual changes in mRNA expression by high throughput sequencing (HTS) and downstream bioinformatics. The processed miRNA sequences were aligned and annotated to known animal miRNAs (miRBase) using the BLAST method. Our data showed that exposure to slick oil resulted in 42, 82 and 254 differentially expressed miRNAs at 24hpf, 48hpf and 96hpf, respectively. In comparison, exposure to source oil resulted in 25, 24 and 167 differentially expressed miRNAs at 24hpf, 48hpf and 96hpf, respectively. Gene ontology (GO) analysis on the target mRNAs was consistent with the pathway analysis of the miRNAs, indicating disruption of cardiovascular system development after slick oil exposure may be through miRNA–mRNA interaction. Slick oil caused an overexpression of miR-133a and miR-15b, which correlated with the decrease in the expression of genes related to the cardiovascular system such as KCNH2 and KLF15. Quantitative real-time PCR will be used to further verify the expression pattern of the selected miRNAs. This work is the first study linking miRNAs and mRNAs in fish responsive to DWH oil exposure, providing a new opportunity for better understanding of the molecular mechanism of DWH oil toxicity. This research was made possible by a grant from The Gulf of Mexico Research Initiative. Grant No: SA-1520; Name: Relationship of Effects of Cardiac Outcomes in fish for Validation of Ecological Risk (RECOVER).

436 Consequences of cadmium-induced epigenetic state on mutation and animal fitness

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There is a pressing need to understand how environmental conditions, including toxicant exposure, influence genome content, structure, and function and, in turn, how individuals and populations cope with changing environments. It is now understood that genomes are more than static, heritable, biological templates, but rather display a wide range of plasticity that is modulated by the environment. These environmental perturbations of the genome are functionally important as we are quickly learning that they contribute significantly to variation in individual physiologies, fitness, dynamics of populations, and influence adverse outcomes. Both base-substitution and structural mutations are known to contribute to genome plasticity. While mutation is a stochastic event, mutation hotspots exist both within and between genomes. The genetic events that give rise to mutation (e.g., altered DNA damage/repair pathways, miscued replication, recombination error, transposable elements) are beginning to be defined in terms of their functions, yet little is known of the mechanisms that destabilize DNA, potentiate mutation, and coordinate mutation within the genome. These studies use *Daphnia pulex*, a model system for environmental genomics, and leverage preexisting mutation accumulation (MA) lines with existing full-genome sequences, developed in the presence and absence of cadmium. Our MA lines provide direct measures of the rate and spectra of neutral genomic mutations and epigenomic modifications, as well as, detection of their contributions to variation in phenotypes. We applied chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-Seq to explore the hypothesis that environmental stress influences epigenetic states including histone modifications, which control access to the genome, and affect organismal fitness by potentiating mutations. Understanding these processes will have profound implications for society and the long-term health of populations, which are living longer in the presence of a large and growing diversity of chemicals that can modify DNA.

437 Transgenerational effects in medaka of developmental exposure to atrazine

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Atrazine is a heavily used herbicide with known effects on endocrine function in vertebrates. Developmental exposure of atrazine can lead to adverse reproductive outcomes, including gonad development. Chemically-induced epigenetic alterations and subsequent transgenerational transmission of adverse effects has been observed with several endocrine disrupting chemicals. However, little information exists on the potential for transgenerational inheritance resulting from early life-stage exposures to atrazine. We investigated phenotypic effects of atrazine on reproductive endpoints in adults (F_0) and subsequent generations (F_2) of medaka (*Orizias latipes*) after developmental exposure. Embryos (F_0) were directly exposed to atrazine (0, 5, or 50 ug/L) during development (1-12 days post-fertilization) with measurements of gonad and liver morphometrics, fecundity, sperm numbers/motility, fertilization rate, and expression of steroid-related genes, and genes important in control of methylation in adults of F_0 and F_2 generations. Measured exposure concentrations were non-detected, 16-22, and 208-223 ng/g-egg in the control, 5, and 50 ug/L exposures, respectively. No changes were detected in growth (weight or length), gonad size, or fecundity in F_0 or F_2 generations. Small, but statistically significant, reductions in sperm parameters and fertilization rates were detected, but not in all treatments. Alterations of steroid pathway genes were evident in both F_0 and F_2 generations; key genes associated with methylation were up-regulated in gonads of F_0 but not F_2 generation. These results indicate that transgenerational phenotypic inheritance from developmental exposure to atrazine occurs. The importance and relevance of these findings to wild fish populations are beyond the scope of this work and will need to be addressed in subsequent studies.

Birds Under Stress – Integrative Studies for Understanding Effects of Environmental Pollution in the Wild – Part 2

438 The use of antimony as a marker of lead shot exposure in American woodcock (Scolopax minor)

A.D. French, Texas Tech University/TIEHH / Environmental Toxicology; W. Conway, Texas Tech University / Natural Resource Management; D. Klein, Texas Tech / Environmental Toxicology

The American woodcock is a webless migratory gamebird whose population has been decreasing since the 1960s. This decline is thought to be due to habitat loss; however, studies have suggested that exposure to high concentrations of lead (Pb) may be contributing to these population declines. Woodcock are susceptible to Pb accumulation through ingesting earthworms, contaminated soil, and direct consumption of Pb shot. Although Pb shot was banned for waterfowl hunting, it remains legal for webless migratory gamebird hunting. To accurately determine the source of Pb that woodcock are exposed to, a trace metal marker was found that would have only originated from Pb shot. Antimony (Sb) is added to Pb pellets in concentrations as high as 6% to make them harder. The high concentrations of Sb in pellets, along with the low background concentrations, make Sb a useful marker of Pb shot exposure. In this study, 196 juvenile woodcock wings were analyzed for Pb and Sb. A positive correlation was found in woodcock feathers and bones, suggesting that Pb and Sb are originating from the same source. We found 50% of the birds with elevated Pb also had elevated Sb concentrations, meaning that these birds were exposed to Pb shot. Future work aims to determine if this relationship exists in other avian species, and to improve the precision of correlations between Pb and Sb. Research to understand the fate, bioavailability and toxicity of Pb to wildlife is becoming an increasingly important topic to ensure proper regulations are in place regarding deposition of Pb shot into the environment.

439 Isotopic signature of lead: A novel approach to understand the habitat use and metal exposure of birds from urban areas?

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Habitat use is increasingly being recognized as an important determinant of contaminant exposure in wildlife. Habitat use and by extension feeding ecology of animals can be documented by coupling GPS-based telemetry with tissue carbon (C) and nitrogen (N) stable isotope ratios. However, for certain urban-adapted species including gulls, the frequent consumption of human-related food may result in isotopic C and N signatures that are difficult to interpret. This is the case for ring-billed gulls, an omnivorous bird that feeds opportunistically in a mosaic of urban and natural habitats in the Montreal area (Canada). Stable isotopes of metals could potentially be used to characterize habitat use and metal exposure of birds because their geochemical cycles are modified by human activities. Studies have linked the isotopic signatures of certain metals such as lead (Pb) to human activities including gasoline usage, paint and hunting (Pb shot). The objectives of this study were to investigate: 1) the relationships between habitat use determined using GPS-based telemetry and isotopic signature of Pb in liver of ring-billed gulls from Montreal area, and 2) the associations between liver concentrations of selected metals and rare earth elements and habitat use of these birds. Preliminary results indicated that Pb isotopic signatures of gulls that forage in different natural or anthropogenic habitats overlapped with each other. Moreover, the time spent in these habitats did not influence the Pb isotopic ratios (Pb^{207}/Pb^{206}, Pb^{208}/Pb^{206}) in gull liver. In contrast, the hepatic concentrations of Pb and Zn positively correlated with the time male gulls spent in solid waste management facilities. Gd, Yb, Er, Dy, Sm, La, Y were all associated with the time male gulls spent in waste-water treatment plant, but not in females. Despite that isotopic signature of Pb was a poor predictor of habitat use of ring-billed gulls, the sources of known toxic metals could be predicted based on time spent in different foraging habitats in the Montreal area.

440 Trace element exposure in tree swallows across the Great Lakes

C.M. Custer, T.W. Custer, P.M. Dummer, USGS / Upper Midwest Environmental Sciences Center

Abstract: Tree swallow, *Tachycineta bicolor,* nestlings were collected from 76 sites across the Great Lakes which included multiple sites at 27 Areas of Concern (AOCs) and 12 sites not listed as AOCs from 2010 to 2015. Livers were harvested and analyzed for 21 elements. Mercury concentrations differed among AOCs, however, concentrations were well within background and generally comparable or lower than most sites outside of the Great Lakes. Mercury does not seem to pose a threat to birds at any of the AOCs monitored. In contrast, selenium (Se) concentrations were elevated at sites on the southwestern shore of Lake Michigan to levels that could be harmful to avian reproduction. Other toxic elements were elevated at selected AOCs, but the concentrations were not at toxic levels. Multivariate analysis identified concentration pattern differences among 8 AOCs that had 3 or more sites sampled. Of 29 possible AOC comparisons, 11 were significant with clear differences in patterns were evident. Concentration patterns of sites within AOCs appeared closely associated with one another. Selenium was the primary contributor to the differences among these 8 AOCs for 9 of the 11 comparisons.

441 Chronic Toxicity of Perfluoroheptanoic acid (PFHpA) and Perfluorooctanoic acid (PFOA) to Northern Bobwhite (Colinus virginianus)

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Perfluorinated carboxylic acids are a chemical class characterized by long carbon chains surrounded with fluorine groups and a carboxylic acid functional head group. They are used for their hydrophobic and non-reactive properties in firefighting foam and other industrial applications. This subset of perfluorinated compounds (PFCs) have been found to be both widespread and persistent in the environment and while these chemicals have been found to accumulate in wildlife, the toxicity of these compounds are not well characterized for non-mammalian vertebrates, including birds. Our study examines the effects of chronic oral exposure of two perfluorinated carboxylic acids, perfluoroheptanoic acid (PFHpA) and perfluorooctanoic acid (PFOA), on growth, development, reproduction, and survival in Northern Bobwhite, a common model species for birds. Data from our studyare being collected to assess toxicity using the survival of exposed adults, egg production of breeding pairs, hatching success, and the growth of chicks over a period of one month. These data will provide both valuable information to establish reliable avian toxicity reference values (TRVs) for these two compounds and will better characterize risk to birds at sites where PFC contamination exists.

442 Analysis of Emerging and Legacy Flame Retardants in Common Tern from the Niagara Migration Flyway using Gas Chromatography Tandem Mass Spectrometry

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Flame retardants are chemicals used in manufacturing to achieve fire safety standards for many products including electronics, household furniture, and machinery. Legacy flame retardants such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) have been phased out due to their endocrine disrupting properties, and have been replaced by emerging flame retardants that include brominated and organophosphorus flame retardants. However, both legacy and emerging flame retardants are persistent and bioaccumulative in the environment. Studies have shown that legacy and emerging flame retardants express toxic effects in both animals and humans. This study focuses on the impact of legacy and emerging flame retardants in common terns (*Sterna hirundo)* which are considered to be a threatened species in New York State. These birds were previously collected from the Niagara River, near the Great Lakes Region. The Niagara River remains vital to the health of Great Lakes common tern population due to its colony sites and feeding areas. Common terns nest on three breakwaters in Buffalo Harbor, as well as various places on the Niagara River. The common tern's diet consists mainly of fish that are directly exposed to water-borne pollutants therefore the common terns are a good representation of how predatory species are indirectly affected by anthropogenic activity. The analysis of both legacy and emerging flame retardants in these birds would provide information on the bioaccumulation potential of PCBs, PBDEs, and the emerging flame retardants in the Great Lakes Region. To extract these lipophilic compounds from freeze-dried brain and livers of the birds, pressurized liquid extraction is utilized, followed by an acidified silica column for lipid clean up. Final extracts are analyzed using a gas chromatograph coupled to a triple quadrupole mass spectrometer. Quantification is based on isotope dilution using 13C-labeled reference standards. This study will provide useful information on the impact of legacy and emerging flame retardants on top predator birds and other species inhabiting the Great Lakes Region.

443 Are damaged erythrocytes linked to reduced activity and self-maintenance behaviors in birds exposed to crude oil?

C. Goodchild, A. Metz, S. DuRant, Oklahoma State University

The extraction and transportation of crude oil poses a risk to wildlife. For instance, the Deep-Water Horizon oil spill released 779 million L of crude oil into the Gulf of Mexico, and caused an estimated 200,000 bird mortalities. In large spillage events, avian population damage estimates are determined by surveying the number of dead and visibly oiled birds. However, these damage estimates do not account for "clean" birds that may have ingested oil by preening oiled feathers or consuming contaminated prey, thus traditional assessment approaches may underestimate avian population damage. Previous studies have suggested that ingested crude oil may damage erythrocytes, thereby limiting oxygen transportation to tissues. However, it is unclear whether damaged erythrocytes affect whole animal performance. In this study, we were interested in whether hemolytic anemia from crude oil ingestion impacts activity and self-maintenance behaviors in birds. To do so, we dosed zebra finches (*Taeniopygia guttata*) daily with control (peanut oil), 3.3 ml/kg, or 10 ml/kg of crude oil. We measured packed cell volume (PCV), hemoglobin concentration, and red blood cell count on days 0, 7, and 15. We also video recorded behaviors during the experiment, and analyzed the videos for a suite of activity and self-maintenance behaviors. We found that exposure to crude oil decreased PCV and mean corpuscular hemoglobin concentration; additionally, crude oil exposure reduced activity, and resulted in a tradeoff between self-maintenance behaviors.

444 Climate Influence on Legacy Organochlorine Pollutants in Arctic Seabirds

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Changing climate can trigger shifts in environmental variables likely to influence the atmospheric and oceanic transport of chemical pollutants into Arctic regions, partitioning and overall fate of these chemicals, as well as their accumulation in Arctic wildlife. It is unclear which, if any, weather or climate variables will have an impact on organochlorine chemical accumulation in Arctic wildlife and on what time scale. We assessed interannual correlations of a suite of weather/climate variables with organochlorine chemical concentrations in the eggs of two seabird species that breed in the Canadian Arctic: the northern fulmar (*Fulmarus glacialis*) and the thick-billed murre (*Uria lomvia*). Organochlorine data for the seabird eggs spanned 40 years (1975-2014), and the influence of weather/climate variables on concentrations was evaluated for time lags of 0 to 10 years. Two approaches to control for the confounding factor of changing historic emissions were evaluated; principal component ordination and truncation. These two approaches allowed us to examine the utility of long versus short time-series and the information gleaned from each. For the full time series (1975-2014), most of the variability in the organochlorine concentration data was related to changes in historic emissions (up to 70.2 % for murres and 77.4 % for fulmars). Controlling for historic emissions, moderate correlations of resulting detrended data with the North Atlantic Oscillation (NAO), atmospheric pressure, and precipitation were found suggesting that under increasingly NAO+ conditions concentrations of certain organochlorine contaminants, relative to others, will increase, dependent on seabird species. The two approaches yielded similar results, however, the longer time series seemed better suited for capturing the effect of broad-scale climate indices such as NAO. This study highlights the interrelated nature of many of the weather and climate variables and the complexity of their impact on chemical accumulation within Arctic wildlife.

445 Induction of Cytochrome P4501A isoforms in cultured liver slices cultured from naïve and pre-treated chicken embryos

J. Head, K. Mittal, McGill University / Natural Resource Sciences; L. Coquilleau, J. Wang, McGill University; J. Brandenburg, McGill University / Natural Resource Sciences

Lipophilic environmental contaminants such as dioxin-like compounds (DLCs) and polycyclic aromatic hydrocarbons (PAHs) can be found in high concentrations in the eggs of wild birds. We are interested in how this early life exposure to contaminants affects sensitivity to subsequent exposures later in life. In the current study, the DLC, tetrachlorodibenzo-*p*-dioxin (TCDD), or the PAH, benzo[*k*]fluoranthene (BkF) was injected into fertilized chicken eggs prior to incubation. At embryonic day 19, livers were harvested and slices of the tissue were grown in culture. Levels of mRNA expression of the metabolic enzyme, cytochrome P4501A (CYP1A), were assessed in each tissue slice. We observed a 50-fold induction of CYP1A mRNA expression in tissues cultured from TCDD- but not BkF-treated embryos. The lack of response in tissues cultured from BkF-treated embryos was likely due to rapid metabolism of the PAH in vivo. Liver slices were also re-exposed to graded concentrations of TCDD or BkF in culture for 24 hours. This re-exposure resulted in dose-dependent increases in CYP1A expression, which varied between tissues from naïve and pre-treated individuals. We are continuing to study this phenomenon in the context of our previous findings relating to PAH-dependent methylation of the CYP1A promoter. DNA methylation of genes involved in xenobiotic metabolism may be useful as biomarkers describing an association between early life exposures to environmental contaminants and sensitivities to subsequent exposures later in life.

PAH Mixtures in the Environment – Identifying Sources and Assessing Risks

446 Trends in Assessing Risks and Evaluating Sources of PAH-Containing Substances in the Environment

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Two decades after the results of animal bioassays became available demonstrating that the oral slope factor for benzo(a)pyrene was 7 to 10 times lower than the value promulgated in 1984, EPA's Integrated Risk Information System (IRIS) program finalized an updated value for BaP. Reasonable risk estimates calculated using BaP as the index compound for the PAHs in a Relative Potency Factor approach will continue to require application of an appropriate bioavailability factor. Recent research shows that different sources of PAH-containing mixtures differ in the availability/accessibility of the PAHs. Biological accessibility of PAHs in sediments is recognized in EPA's 2003 equilibrium partitioning guidance, which has been proven to be more predictive of potential sediment toxicity to aquatic biota than the 1990s-era consensus standard. Systematic reviews of the many studies of occupational exposure to PAH-containing materials suggest that hazard characterizations of PAH-containing materials should be revisited to reevaluate appropriate cancer classifications under environmentally relevant conditions. These developments – some decades old - in assessment of both human health and ecological risks should reduce the perception of high risk that was fueled by old anecdotal evidence from Percival Pott to the industrial revolution. Remarkably, however, PAHs and PAH-containing materials have recently been included on some lists of "Emerging Contaminants," as if little is known about what are among the most studied compounds in toxicology and the environmental sciences. A similar phenomenon can be seen in the use of PAH source identification methods seemingly to bolster the "Emerging Contaminants" theme. The field of PAH forensics began as an effort to assist in apportioning responsibility among potential local sources. Research investments were made to develop source determination methods that meet legal standards of evidence. Scientific evaluation of graphical, statistical, and mass balance modeling methods of PAH source identification and apportionment should adopt foundational forensic research as a point of departure for further research rather than uncritical adoption of selected techniques from the methodological toolbox as "plug and play" black boxes to be applied with incomplete understanding of potential sources and source variability.

447 Equilibrium sampling indicates increase of thermodynamic potential of PAHs during sludge digestion due to matrix depletion

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Sewage sludge, the end-product from waste water treatment plants (WWTPs), is often used as soil fertilizer. At the same time, it is a potential source of contaminants such as PAHs to soils and aquatic environments. PAH contamination is often assessed based on total concentrations, determined by exhaustive extractions, which however do not provide any information on effective concentrations for biotic uptake, transport processes or transformation processes. Freely dissolved concentration is in terms of exposure a more meaningful parameter to investigate. Our study provides a new approach for investigating freely dissolved concentrations of PAHs in WWTP sludge by applying equilibrium sampling. The objectives of the study were: *i*) validation of the methodology on WWTP sludge, *ii*) measurement of "activity ratios" to quantify differences in the thermodynamic potential of target PAHs between and within WWTPs, *iii*) comparison of PAH exposure levels in sludge from Danish WWTPs with sediment levels from the Baltic Sea. Secondary and digested sludge were obtained from three Danish WWTPs. Biological activity was prevented by sodium azide and then equilibrium sampling was conducted in jars coated on the inside with multiple thicknesses of silicone. After equilibration, the silicone was extracted. Without further treatment than addition of internal standard, the extracts were analysed using GC-MS EI. The equilibrium concentration of PAH in silicone ($C_{silicone}$) was obtained from the linear relationship between mass of PAH and mass of silicone. Freely dissolved concentrations (C_{free}) were calculated from $C_{silicone}$ and analyte specific silicone-water partitioning ratios, and these C_{free} values were very similar to or 2-3 times higher than measured in Baltic Sea sediment. The chemical activity ratio (AR) was determined as ratio of $C_{silicone}$ between secondary and digested sludge. AR expresses the difference in thermodynamic potential of the PAHs before and after sludge digestion. For all measured PAHs, including alkylated phenanthrene, the measured AR>1 in all three investigated WWTPs and the tendency was that AR decreased with increasing hydrophobicity. The increase in chemical activity or freely dissolved concentration when going from secondary to digested sludge is probably caused by "solvent depletion" - reduction of sorption capacity during the digestion.

448 Factors controlling human exposures to PAHs in soil and methods for assessing bioavailability

Y. Lowney, Alloy, LLC / Health Sciences

Several studies reported in the literature suggest that interactions between PAHs and soil diminish PAH bioavailability and thus reduce risk from incidental ingestion of PAH- contaminated soil. This presentation will provide an overview of a broad, multi-organization research effort to explore the influence of soil composition, PAH concentration, and PAH source material type on PAH bioavailability to humans, using an approach capable of measuring uptake at low, environmentally-relevant PAH concentrations (down to 1 ppm or less). This research program, funded by the Strategic Environmental Research and Development Program (SERDP), evaluated the chemical controls on the oral bioavailability and dermal absorption of PAHs in soil. Major findings of the effort are that the relative oral bioavailability of benzo(a)pyrene (BaP) was observed to be strongly dependent on PAH source material and ranged from about 20% (PAHs in black carbon matrices) to 90% (PAHs in fuel oil). Dermal absorption of BaP was not observed to be dependent on soil composition for the four soils tested (different PAH source materials were not tested) and was lower than USEPA's current default value for dermal absorption of BaP. Effort from this study also indicate that rapid and inexpensive in vitro methods show good promise for predicting the oral RBA of PAHs from soil. The implications of these data for human health risk assessment will be discussed, along with their potential impacts on soil cleanup goals.

449 States' Approaches to Assessing PAH Risks

B.H. Magee, N.D. Forsberg, ARCADIS

USEPA released four new toxicity values for benzo(a)pyrene (BaP) in January 2017. USEPA has concluded that BaP is 7.3-times less potent as an oral carcinogen than previously thought. This change in the oral slope factor will increase all cancer risk-based criteria for soil and water by 7.3-fold. The new toxicity values will affect all risk-based criteria and standards for BaP and other PAHs that are included in the calculation of benzo(a)pyrene-toxic equivalents (BaP-TE). USEPA had initially proposed an onerous dermal slope factor, but the final value was delayed pending further work, as has USEPA's final relative potency factor approach for PAH mixtures that proposed a longer list of PAHs of concern. The new values, which are based on both cancer and non-cancer effects, are already being used as defaults in USEPA's Regional Screening Level (RSL) calculator even though the RSLs have not been updated as of June 2017. With the uncertainties concerning USEPA's program priorities, many remedial decisions for PAH sites will fall to the state regulatory agencies. Massachusetts, Michigan, Florida, and Texas are using the new toxicity factors in their programs. Virginia is waiting for the RSL tables to be updated and will use the values in the updated tables when available. These states are using the standard list of seven potentially carcinogenic PAHs to calculate BaP-TE concentrations. To more fully understand the states' approaches, a survey will be presented that shows the PAH approaches being used in other states. The results of this survey will be updated immediately prior to the meeting to ensure that the results are timely.

450 PAH Source Identification in Urban Settings: Avoiding the Sin of Oversimplification of Pyrogenic Sources

J. Pietari, Exponent, Inc. / Environmental Sciences; T. Saba, Exponent, Inc.; P.D. Boehm, Exponent, Inc. / Environmental & EcoScience Group

Polycyclic aromatic hydrocarbons (PAHs), both from legacy industrial operations and present-day sources, are ubiquitous environmental pollutants that necessitate remediation of soils and sediments worldwide. While differentiating among petrogenic, pyrogenic, and biogenic contributions using specialty analytical techniques for an extended "forensic list" of PAHs, biomarkers, and saturated petroleum hydrocarbons is common practice, determining contributions from varying yet quite similar pyrogenic sources is much more challenging. Pyrogenic PAHs affecting urban environments can come from roofing materials, smelting operations, creosoting, leaching from creosoted pilings in aquatic sediment sites, pavement sealants, diesel soot, and the manufacture of gas or tar-based products such as Tarvia. The common practice of relying solely on one or two simple diagnostic ratios of priority pollutants is subject to large error and is therefore insufficient to characterize the sources of pyrogenic PAH impacts in the environment. Additionally, use of principal component analysis (PCA), an exploratory statistical analysis, to apportion PAH sources without including all plausible sources is nearly impossible in complex urban settings. These two factors are particularly important when allocating or apportioning costs for remediating urban sediment sites. Thus, PAH source identification based on the chemistry of sediments in urban settings in which similar pyrogenic sources are mixed must go hand in hand with robust evaluation and chemical characterization of historical and current potential PAH sources. Through a case study, we illustrate the contributions of 1) oversimplification of pyrogenic sources and 2) over-reliance on limited forensic tools (namely priority pollutant diagnostic ratios and PCA) to misidentification of PAH sources and the implications to allocating/apportioning costs associated with PAH-contaminated sites.

451 Characterization of PAHs in Surficial Sediments- Urban Background and Evidence for Industrial Impacts in New York City Waterbodies

S. Gbondo-Tugbawa, E.A. Garvey, C. Prabhu, Y. Wang, Louis Berger, Inc.; R. Weissbard, E. Mahoney, New York City Department of Environmental Protection

The occurrence of PAHs has been suggested as major factor driving toxicity in the NY/NJ harbor sediments. However, PAH concentrations in the harbor vary by orders of magnitude, likely reflecting a wide range of sources such as industrial discharges or the local degree of urbanization. The City of New York has been named as a possible responsible party for two Superfund sites, Newtown Creek and Gowanus Canal, where PAH contamination is extensive and many industries exist side-by-side with municipal discharges. Baseline data obtained as part of the Newtown Creek Superfund site investigation indicates that sediments in waterbodies free of industrial inputs generally exhibit low levels of PAHs, typically averaging less than 60 mg/kg PAHs (the sum of 44 parent and alkylated daughter compounds). We assert that concentrations at this level essentially comprise "urban background" and do not pose significant impacts to human health or the environment. Supporting our premise, studies of sediment toxicity in 14 urban background water bodies sampled as part of the Newtown Creek superfund site investigation generally exhibit little or no toxicity at these PAH concentrations (see J. Cura *et al.,* this conference); and in general, Superfund regulations do not require cleanup actions to concentrations below natural or anthropogenic background levels. Sources of PAHs to NYC harbor sediments are quite varied and include major industrial discharges associated with manufactured gas plants (MGP sites) as well as petroleum refining and handling. Municipal discharges of storm water and combined sewer overflows (CSOs) have also been thought to be substantive sources. In this presentation, we will present evidence to show that waterbodies with high levels of PAHs in the sediments are associated with industrial sites. For waterbodies where only municipal discharges (stormwater and CSOs) are present, sediment levels of PAHs are low, as is sediment toxicity. To further this point, the presentation will include a study of PAH patterns and concentrations in Gowanus Canal, contrasting municipal discharge PAH patterns with those of nearby MGP waste oil and those of the sediments of the canal itself. Observed deviations from the established background PAH concentrations, the proportion of pyrogenic PAHs, PAH source ratios and profiles can be used on sites like Newtown Creek and Gowanus Canal to indicate the presence of upland sources of PAHs superimposed on the urban background.

452 Polycyclic Aromatic Hydrocarbon Accumulation in Soil Receiving Rooftop Runoff

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Polycyclic aromatic hydrocarbons (PAHs) are persistent organic pollutants appearing ubiquitously in the environment with potential impacts on human health. The main objective of this study was to qualify and quantify PAH accumulation (especially carcinogenic PAHs (cPAHs)) throughout Oklahoma City while investigating factors related to higher accumulation: building use (residential, commercial, and school) and roofing type (asphalt, metal, and tar). To determine if cPAH concentrations were higher in soil receiving direct rooftop runoff (contact samples), paired samples were evaluated from each site. A digestive model was applied to indicate bioavailable fractions after soil ingestion. A majority of locations analyzed had levels of cPAHs above the USEPA's soil screening level (SSL) including benzo[a]pyrene (BaP), which was representative of the cPAH concentrations. Runoff contact samples surrounding schools had the highest significant values of cPAH contamination within building usage. Schools and commercial contact soils had significantly elevated levels of cPAHs and BaP as compared to residential contact soils. Roofing did not vary in contributing to cPAH levels. cPAH concentrations in contact soils were significantly greater within each sampling subset compared to the paired reference sample. The digestive model indicated less than 3% bioavailability. Sites were also evaluated for potential correlation of contamination with neighborhood income levels. Because many highly concentrated cPAH soils were found in school areas, school sites in particular should be further investigated for contamination. Despite overall low bioavailability, the magnitude of cPAH concentrations in soil is still a cause for concern.

453 Application of Receptor Models to Characterize Sources of PAHs in Urban Sediment: Use of Site Specific versus Generic Source Profiles

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Receptor models, such as EPA's Chemical Mass Balance (CMB), are used to evaluate potential sources of polycyclic aromatic hydrocarbons (PAH) in watersheds and sediments. Mathematically these models mix the chemical profiles of potential source inputs in various combinations to find the best match with the profile of each environmental sample. When used correctly, they provide information on the relative contributions of various sources. Challenges include identifying an appropriate set of sources and properly characterizing their PAH profiles. At legacy sites examples of historic source materials are often not available. Chemo-statistical approaches such a concentration-weighted averaging of site samples know to be impacted by particular sources and iterative application of principal component analysis and CMB can be used to identify technically defensible source profiles. Over the past 15 years there has been an increasing use of literature derived generic source profiles when modeling sources of PAHs in lakes and rivers. Such use of generic source profiles raises a number of technical concerns. Literature-derived source profiles may be appropriate if variability within a source type is low, and the range of profiles does not overlap with other potential sources. But, averaging a number of distinct profiles can generate an aggregate profile that is not consistent with any actual source. Evidence on how well the aggregated profiles represent the claimed site-specific sources is rarely presented. It is well known that variations in combustion conditions and post emission weathering can result in large differences in the PAH profiles further limiting the usefulness of generic profiles. Using case studies, this presentation will compare the application of receptor models within a single waterbody using profiles developed from site data to applications that attempted to evaluate sources of PAHs in urban sediments in different aquatic systems using a set of generic profiles. Tools for evaluation and validation of source profiles will be discussed.

Screening and Prioritization Methods for Characterizing Risk of Contaminants in the Environment

454 Ecological Risk Classification and Assessment of Inorganic Substances under the Third Phase of the Chemicals Management Plan

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Under the *Canadian Environmental Protection Act, 1999,* Environment and Climate Change Canada and Health Canada assess and manage, where appropriate, risks of chemical substances to the environment and to human health. The Chemicals Management Plan (CMP) is a Government of Canada initiative that addresses approximately 4300 substances identified as priorities for assessment. In the third phase of the CMP (2016-2020), about 1550 substances are being addressed, including approximately 380 inorganic substances. Early activities to address remaining inorganic substances include identifying data needs, developing tailored strategies and novel approaches, and preliminary stages of assessment drafting. A relative risk approach is under development for identifying and classifying the potential ecological risks of inorganic substances. Two key lines of evidence were used for this approach. Modelled environmental concentrations were determined using data from the Domestic Substances List inventory update (DSL-IU) and National Pollutant Release Inventory (NPRI) combined with conservative exposure scenarios. Measured environmental concentrations are determined from surface water quality data from Canadian monitoring and surveillance programs. The relative ecological risk of individual inorganic substances and groups was determined by comparing predicted and measured concentrations to selected predicted no-effect concentrations to determine an overall preliminary risk classification. The ecological risk classification of inorganic substances is a novel and efficient method allowing Environment and Climate Change Canada to focus ecological assessment efforts on remaining substances of higher ecological concern. Recent updates to preliminary ecological risk classifications for inorganic substances will be presented. Updates on the CMP and status of current ecological assessments of inorganic substances will also be provided.

455 Differentiating pathway-based toxicity from non-specific effects in high-throughput data: A foundation for prioritizing targets for AOP development

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The ToxCast chemical screening approach enables the rapid assessment of large numbers of chemicals for biological effects, primarily at the molecular level. Because adverse outcome pathways (AOPs) connect biomolecular effects with potential adverse outcomes at the level of the individual or population, they offer a means to support chemical hazard assessment using ToxCast data. Arguably, AOP development is most critical in relation to specifically-acting toxicities for which effective structure-based prediction models are currently lacking. Previous work identified a ‘cytotoxic burst’ phenomenon wherein large numbers of the ToxCast assays begin to respond at or near concentrations that elicit cytotoxicity. The concentration range over which the burst occurs is statistically definable. We conducted a meta-analysis to identify which assays were frequently responding at concentrations well below the cytotoxic burst and are, ostensibly, providing the most pathway-specific effect information. Assays were ranked by the fraction of chemical hits below the burst concentration range compared to the number of chemicals tested, as well as by diagnostic-odds ratio, resulting in a ranking of potentially important, target-specific ToxCast assays. The assay prioritization based on the cytotoxic burst definition indicated numerous assays with targets previously identified for AOP development (e.g., thyroid peroxidase, peroxisome proliferator-activated receptor γ, estrogen receptor α, aromatase) along with several novel targets (e.g., progesterone receptor, monoamine oxidases A and B, norepinephrine transporter). Additional analyses identified chemicals that elicited assay activity only at concentrations within the cytotoxic burst region suggesting a non-specific mode of action. These chemicals were anticipated to be classified as likely to elicit toxicity largely through narcosis, which was largely supported when the chemicals were cross-referenced with other Mode of Action classification/prediction tools (e.g., Assessment Tools for the Evaluation of Risk; ASTER). These analyses help to differentiate which pathway-specific assay targets to prioritize for AOP development. They also provide insight into the utility of tools which integrate chemical occurrence data with the high-throughput screening data, such as the USEPA’s Exposure Activity Ratio Calculator. The content of this presentation neither constitute nor necessarily reflect USEPA policy.

456 Test driving new tools for monitoring contaminants of emerging concern in California waterbodies

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The introduction each year of thousands of new chemicals into commercial and consumer products necessitates a modernization of current monitoring practices for ambient water quality, which often miss the occurrence and deleterious effects associated with contaminants of emerging concern (CECs). To evaluate the utility of high throughput cell-based “bioscreening” assays and non-targeted chemical analysis, water and sediment samples from rivers and streams across California were collected and analyzed using these new monitoring tools. Ambient water samples were subjected to solid phase extraction (SPE), concentrated, solvent exchanged and analyzed using a standardized suite of bioscreening assays. In parallel, aliquots of SPE extracts were analyzed by LC-MS/MS and GCxGC-TOF/MS using both targeted and non-targeted approaches. The bioscreening response for endocrine active chemicals in these waterways was minimal, suggesting a low potential for reproductive effects on fish. In contrast, bioscreening for dioxin-like chemicals was frequently above detection limits and more widespread in southern California streams, particularly those dominated by urban land use. Preliminary non-targeted analysis indicated the presence of several possible bioactive chemicals which are not routinely addressed by regional monitoring programs. These initial pilot evaluation results show that bioscreening and non-targeted analysis are promising elements in modernizing water quality monitoring for CECs.

457 A screening and prioritization strategy for contaminants of emerging concern in California watebodies

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Collectively known as contaminants of emerging concern (CECs), substances that occur in ambient waters but for which little effects-based information is available, continue to pose a challenge for water quality managers. Based on the recommendations of a panel of independent experts, a framework to screen for a broad suite of CECs, and to identify the most problematic chemicals is being developed and test driven for waterbodies in California. This framework combines a traditional risk-based element for chemicals for which sufficient occurrence and effects data is available, with new tools that screen for chemicals sharing common modes of biological activity and that identify bioactive CECs using non-targeted chemical analysis. Meant initially to supplement monitoring of individual chemicals, cell-based transactivation assays (“bioscreening”) provide an additional advantage of informing which whole animal endpoints are most likely to be impacted. Where targeted chemical monitoring fails to identify a likely bioactive/causative agent, non-targeted mass spectrometry is employed to broaden the universe of chemicals analyzed. Expanding the bioscreening toolbox to address the most relevant CECs and their adverse outcome pathways, and establishing quantitative linkages among ambient levels of CECs, bioscreening responses and whole animal effects thresholds are the next key steps in solidifying the utility of this framework.

458 Screening level benchmarks — providing risk-based prioritization for environmental contaminants with minimal toxicity data

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Tens of thousands of chemicals are used, produced, and potentially released into the environment. The Minnesota Department of Health (MDH) is tasked with assessing human health risks from exposure to contaminants in drinking water. Risk-based prioritization is often used to make informed decisions about which contaminants to focus on for monitoring studies and development of guidance values. Unfortunately, the data available for toxicity and exposure evaluations is often not uniform across chemicals, making unbiased risk-based determinations difficult. This is particularly true of chemicals of emerging concern, which frequently have little to no available toxicological data. Due to the increasing number of chemicals being introduced into the market, expanding analytical capabilities for detecting chemicals, and the cost of conducting toxicity studies there is a clear need to identify alternative methods for providing risk context for contaminants that have insufficient data to perform a traditional risk assessment. MDH, with funding provided through the Clean Water Fund of the Minnesota Clean Water, Land and Legacy Amendment, compiled a database of toxicity values for hundreds of chemicals from multiple sources and tested screening level methods to assess uncertainties and limitations of each method. Testing included an evaluation of each method’s capacity to address a wide variety of adverse health endpoints (e.g., developmental, cancer) and substances (e.g., solvents, pharmaceuticals, pesticides) as well as the time and expertise required. Preliminary testing confirmed that available methods are not adequate to address inorganics, radionuclides, nanomaterials, high molecular weight polymers, highly bioaccumulative compounds, or chemicals specifically designed to have hormonal activity. Testing results identified the LD50 extrapolation and the toxicological threshold of concern (TTC) methods to be capable of producing screening values that were adequately protective as compared to traditionally derived guidance values. While these methods are not intended to replace traditional toxicological-based risk assessments, they may be useful tools for providing health protective screening values that can be used to prioritize further assessment. Testing results as well as a comparison of the methods will be presented along with a discussion of the limitations and tools for identifying chemicals for which these screening methods did not work well.

459 Communicating Risk Assessment: The RISK21 Framework and Web-Based Tool

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An integrated risk assessment framework that enhances efficiency and risk management was developed by the HESI Risk Assessment in the 21st Century (RISK21) project. The project was initiated to develop a scientific, transparent, and efficient approach to the evolving world of human health risk assessment, and resulted in the development of a framework that reconsiders the way chemical risk assessment information is obtained and used. It is a problem formulation-based, exposure driven, tiered data acquisition approach that allows an informed decision on safety to be made when sufficient evidence is available. The RISK21 approach maximizes the ability to inform decisions and optimize resource usage, and the program also developed a web-based tool that allows users to easily communicate risk-based decisions, whether for a screening and prioritization purpose or a definitive risk assessment. This tool is freely available at www.risk21.org. An illustrative case study demonstrated the application of this approach by evaluating a large number of chemicals potentially present in drinking water. Chemicals were prioritized and evaluated to determine which are of highest potential concern for human health risk assessment. Out of an initial list of 20 potential drinking water contaminants, four were prioritized for additional assessment based only on low-tier exposure and hazard estimates and/or already available information. Using this approach, the list of chemicals was reduced by 55% by using exposure estimates and low tier toxicity estimates, and by another 25% by using data from existing toxicology studies, while using no additional animals. The utility and uptake of this approach and the web-tool has been demonstrated via several hands-on case study workshops, led by RISK21 team members and hosted or sponsored by various groups, including government agencies. These workshops have engaged participants in real-world case examples, and combined, have already reached over 500 people with direct, hands-on use and application of the web-tool in the US, China, Taiwan, Brazil, and Canada, with additional workshops planned. Although work to-date has focused largely on human health risk assessment applications, the approach and the associated web-tool is broadly applicable across disciplines for risk assessment, prioritization, communication, and outreach.

460 A Hazard Based Assessment Approach for Chemical Products/ Commercial Formulations - Application of GHS Mixture Rules

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Human health and environmental fate and toxicity tests are mainly performed to evaluate hazards of individual substances. For chemical mixtures, this is a difficult process, as testing mixtures is logistically complex and costly. In addition, testing will not identify specific chemicals responsible for the response. The Globally Harmonized System of

Classification and Labelling of Chemicals (GHS) provides a systematic approach for the health hazard evaluation of a chemical mixture based on its components. GHS is in force in 72 countries around the world, including the U.S. This approach is complex and assumes that the effects of individual compounds are essentially additive. It involves the use of cutoffs or concentration limits and requires calculations for a number of hazard endpoints. Further, it requires detailed information regarding identity, concentration and toxicity of individual compounds. Towards this, we utilized GHS mixtures rules to assess hazards of several chemical formulations used in the textile industry. In our approach, human health and environmental hazards associated with individual chemicals present in the formulation (at concentrations of 100 ppm and above) were assessed using a combination of hazard screening tools, including USEPA's Safer Choice Program (SCP) Functional Class Criteria, the USEPA SCIL, and Clean Production Action's GreenScreenÒ for Safer Chemicals. Then, GHS mixture rules were applied to assess the hazard of the whole formulation on 16 human health and environmental hazard endpoints such as carcinogenicity, skin sensitization, and flammability. This approach can help formulators identify which ingredients within a commercial formulation are hazardous and are good candidates for substitution or elimination. The work provides an overview of our GHS hazard-based approach, and illustrate the paradigm with examples on commercial chemical mixtures.

461 Complex Mixtures: From Hazard Assessment to Risk Assessment

H. Plugge, 3E Company

Most current decision trees regarding the greenness of commercial chemicals overemphasize hazard assessment, i.e. little attention is paid to the exposure aspects and hence the actual risk incurred. In addition most hazard assessment methodologies suffer from an inability to assess more than one chemical at a time. Given that most commercial products are mixtures of multiple chemicals or single chemicals with significant impurities, this severely limits the applicability of most hazard assessment methodologies. Hence the need exists for the ability to screen the hazards from both single chemicals and highly complex mixtures. In addition risks could be screened via modeled direct exposure estimates or through surrogates such as emissions or inventory, as risk = exposure * hazard. Our methodology is driven by raw scientific data both direct and imputed. Data curation and user supplied data are integral to the methodology. Raw data from 20+ endpoints are transformed into scores between 1 and 1000, 1000 being the greenest. Because the hazard screening scores are dimensionless transformations, they can be summed through weighted geometric means across chemicals. Complex products/mixtures thus receive a single hazard score with a clear delineation of the contribution of each chemical to the overall hazard screening score. To derive a risk screening score an exposure screening measurement needs to be derived. For volatile chemicals this could be an exposure concentration approximated via relatively simple exposure models. For more complex assessments one can use facility emissions or inventories as exposure surrogates/proxies thereby deriving facility wide risk scores. Alternatives assessment is thus greatly facilitated: unrelated chemical mixtures can easily be screened /scored against each other including allowing for what-if analyses of possible alternative compositions, especially those based on existing chemicals. For chemicals without known data, trend analyses across matrices can provide additional data.

Remediation and Restoration – Assessing and Measuring Effectiveness for Contaminated Sediment

462 Developing Sediment Remediation Goals at Superfund Sites Based on Pore Water for the Protection of Benthic Organisms from Direct Toxicity

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Passive sampling is becoming a frequently used measurement technique at Superfund sites with contaminated sediments. Passive sampling measures the concentrations of freely dissolved chemicals (C_{free}s) in the sediment pore water. C_{free} has been found to be a very practical means for estimating the concentrations of bioavailable chemical in the sediments. Building from this approach, a methodology has been developed to derive sediment Pore Water Remediation Goals (PWRGs) for the protection of benthic organisms from direct toxicity using C_{free}s measured with passive sampling. The methodology builds upon USEPA's Equilibrium Partitioning Sediment Benchmarks (ESBs) approach where ESBs are derived using the product of the organic carbon normalized sediment-water partition coefficient (K_{OC}) and the Final Chronic Value (FCV) from USPA's ambient water quality criteria for the protection of aquatic life. In the developed methodology, ESBs are used as a screening level to determine where passive sampling measurements should be performed at the site. With passive sampling measurements, site-specific K_{OC} values are determined and subsequently, used with USEPA's FCVs to estimate risk to benthic organisms. If unacceptable risks to benthic organisms exist, PWRGs are developed for the site. The PWRGs may be expressed on $C_{free:PWRG}$ (µg/L), $C_{SOC:PWRG}$ (µg/kg-OC), or $C_{S:PWRG}$ (µg/kg-dw) bases. The PWRG approach should also facilitate comparison of sediment toxicity data to sediment chemistry, by indexing chemistry to bioavailability (C_{free}). The methodology will be presented along with illustrative examples and published as USEPA guidance (USEPA 600/R-15/289). This abstract does not necessarily reflect USEPA policy.

463 Equilibrium status of aquatic organisms with different functional traits exposed to PCB contaminated sediment treated with activated carbon

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In situ remedies for contaminated sediments typically offer rapid risk reduction but ideally must sustain that reduction over the long-term via resiliency to processes that can compromise or degrade the remedy's benefits (e.g., through recontamination of surface sediments). One of the focus of our study was to evaluate the effects of contaminant influx on the performance of activated carbon (AC) amendment, while also determining bioaccumulation by three marine organisms that interact differently with a PCB-contaminated sediment (New Bedford Harbour, MA, USA) in 90-day mesocosm experiments. AC amendment (4.3% by mass) mixed into the bed sediment for 1 month was compared to unamended sediment. Bioaccumulation was measured in *Nereis virens* (worm), *Mercenaria mercenaria* (clam) and *Cyprinodon variegatus* (fish). Sediment and overlying water were monitored using polymer sampling: *ex situ* equilibrium sampling with multiple thicknesses of silicone, and in situ pre-equilibrium sampling with polyethylene (PE) corrected for equilibrium using performance reference compounds. The AC remedy provided >90% reduction in total native PCBs in worms, clams, and fish, similar to the >90% reduction in silicone and PE, demonstrating that polymers reflected the remedy efficacy in reducing bioaccumulation. However, reductions of highly hydrophobic PCBs by AC detected by the polymers were accompanied by no reduction in bioaccumulation. Kinetic limitations and residue AC in the gut are the suspected causes for the apparent limitation of AC to reduce the bioaccumulation of highly hydrophobic congeners. Concentrations in lipid when in thermodynamic equilibrium with the sediment as determined by equilibrium sampling results were compared to lipid-normalized PCB concentrations in the organism to assess bioaccumulation equilibrium status relative to the sediment. All organisms were far below the thermodynamic potential for bioaccumulation, with the worm lipids being closest at about 10% of the potential. This general under-equilibration is likely, at least in part, due to exposure to overlying water and fish and clam food that were far below thermodynamic equilibrium with the sediment. Influx of contamination was frequently added, as sediment spiked with congeners not present in the bed sediment (input congeners), during the 90-day experiments. Overall, AC amendment reduced bioaccumulation of most input congeners across all species in agreement with previous studies.

464 Evaluation of DDT Availability in Sediment Following Placement of a Thin-Layer Cap in Quantico Embayment

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Enhanced monitored natural recovery (EMNR) is a sediment remedy that accelerates natural recovery processes by placing a relatively thin cover of material over contaminated sediment areas. EMNR can be a cost-effective alternative to conventional remedies such as capping and dredging. We evaluated the performance of a thin-layer habitat enhancement sand cap (TLC; target depth 6-12 inches) to reduce bioavailable concentrations of organochlorine pesticides (e.g., 4,4'-dichlorodiphenyltrichlorethane [4,4'-DDT], 4,4'-dichlorodiphenyldichloroethane [4,4'-DDD] and 4,4'-dichlorodiphenyldichloroethylene [4,4'-DDE]) in sediment, at Site 99 Quantico Embayment adjacent to Quantico Marine Corps Base, Quantico, Virginia, USA. Performance monitoring included in situ and *ex situ* bioaccumulation and porewater measurements to assess changes in pesticide bioavailability over time: tissue concentrations were obtained via two-week deployments of two species, *Lumbriculus variegatus* (oligochaete worm) and *Corbicula fluminea* (Asian clam); solid phase microextraction (SPME) sampling was used to measure porewater from 0 to 24 inches below the sediment-water interface and at multiple depth intervals within and below the TLC. Surface sediment (top 7 cm below the SWI) were an average of 974 µg/kg dry weight (dw) from baseline monitoring in 2012, which decreased in short-term 2014 post-cap monitoring (210 µg/kg, dw), and continued to decrease in the first and second annual long-term post placement monitoring events, 104 µg/kg (dw) after 14-months, and 51 µg/kg (dw) after 25-months. *L. variegatus, C. fluminea*, and porewater measurements showed sustained, and in most cases, statistically significant reductions in total DDTs (DDX) ranging from 67-86%, 25-55%, and 30-61%, respectively, during the 25-month post-remedy monitoring period. We observed strong correlations between concentrations of DDX in *L. variegatus* tissue and surface sediment porewater (r^2 = 0.82 to 0.95). Relatively poor correlations, however, were observed between concentrations of total DDX in *C. fluminea* tissue and surface sediment porewater (r^2 = 0.28 to 0.16). We attribute the poorer correlation for clam tissue to the filtering feeding behavior of the clam relative to the deposit feeding oligochaete.

465 Development of a Baseline and Long-Term Monitoring Plan for the Portland Harbor Superfund Site

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Baseline and long-term monitoring programs provide an objective basis for evaluating remedy performance and effectiveness of sediment remedies at achieving remedial action objectives (RAOs). Baseline monitoring is to assess the conditions at the site prior to construction and serves as the basis for comparison to data collected during the long-term monitoring program. Long-term monitoring takes place during and following implementation of the remedy and continues until the remedy has met the established goals. The long-term monitoring program compares Site conditions against baseline conditions and cleanup levels to assess the effectiveness of the remedy. Long-term monitoring does not include performance monitoring of specific remedial measures such as monitoring cap integrity but rather focuses on whether the site remedy is achieving the RAOs established for the site through a comparison to risk-based, ARAR-based and background-based cleanup levels. EPA issued its record of decision (ROD) for the Portland Harbor Site in Portland, Oregon in January 2017. The selected remedy include a combination of dredging and excavation, capping, in-situ treatment, enhanced natural recovery and MNR. Subsequent to the ROD, a framework for a comprehensive baseline and long-term monitoring program has been developed. The monitoring program includes surface sediment, sediment traps, surface water, and biota tissue for a range of species. Surface water will be monitoring using both high volume sampling approaches and semi-permeable membrane devices (SPMDs). Biota tissue targeted for sample collection include carp, smallmouth bass, crayfish, clams and osprey eggs. Monitoring of migratory and anadromous fish tissue will also be collected to support the 5-year review process and the update of fish consumption advisories. The required number of samples for the monitoring program were estimated using statistical procedures that considered the expected decline in contaminant concentrations and the variability in the data set. We will describe the approach for development of the baseline and long-term monitoring program including t stakeholder involvement. We will describe the monitoring objectives, boundaries, elements and timeframe the monitoring program. We will also describe the process for evaluating remedy effectiveness including a comparison to site cleanup levels and target tissue levels and an equivalency evaluation that compares site data to upriver data.

466 PCB Tissue Concentrations and Benthic Community Impacts at a Carbon Amendment Pilot Study in the Intertidal and Subtidal Zones of San Francisco Bay

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Historical site activities at the Hunters Point Naval Shipyard (HPNS) in South San Francisco Bay resulted in the release of chemicals, including polychlorinated biphenyls (PCBs), to offshore sediments. To inform remedy selection at this urban site, activated carbon (AC) amendments alternatives were evaluated in a pilot treatability study. Two 0.4 acre plots extending from the intertidal to the subtidal zone were treated with either AquaGate + PAC™ or SediMite™ were assessed for their potential to reduce ecological risks associated with PCB-contaminated sediment. Previous treatability studies indicated that AC may be effective at reducing the bioavailability of PCBs to the bent-nose clams (*Macomanasuta*) in shallow intertidal sediments when aided by mechanical mixing. This study assessed the effectiveness of AC placements without mechanical mixing in deeper water that is more representative of conditions where full-scale remediation is expected. Tissue bioaccumulation, benthic invertebrate community composition, and chemical analyses were measured as indicators of remedy effectiveness. Comparisons were made between baseline, reference, and post-amendment conditions (8 and 14 months post-placement). PCB tissue concentrations in *Macoma* sp. were measured in situ (field) and *ex situ* (bench-top) after 28-day exposures. Developing field exposure chambers that allowed sediments to infiltrate the chambers and expose clams upon deployment and then retrieve the sediment and exposed organisms for chemical analyses was a challenge. Modifying a chamber design used in previous studies by Luthy et al. (2009) proved successful. Test organisms were another challenge. Tissue bioaccumulation was planned to be conducted with *M. nasuta* but instead, initial measurements were made with *M. secta* (white sand clam) collected at a nearby reference location where *M. nasuta* had been previously found. The species have a similar appearance and life histories but *M. secta* had low survival in the field (< 20%), lab exposures (< 60%), and lab controls (10%). Additional field pilot testing led to the use *M. nasuta* from a supplier for post-amendment monitoring. PCB tissue concentrations were reduced by up to 85% in both pilot amendment areas after 14 months with clam survival greater than 90%. Benthic invertebrate communities in test plots were not significantly different from baseline conditions or among treatments 14 months after AC deployment.

467 Assessing sediment quality in the Great Lakes Connecting Channels using National Coastal Condition Assessment protocol

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The EPA Office of Water's National Coastal Condition Assessment (NCCA) helps satisfy the assessment and antidegradation provisions of the Clean Water Act by estimating water, sediment, and benthic quality conditions in the Great Lakes nearshore on a five-year cycle starting in 2010. Beginning in the 2015 NCCA cycle, the connecting channels of the Huron-Erie corridor (HEC; 2014, 2015) and St. Marys River (SMR; 2015, 2016) were assessed through a partnership with Great Lakes National Program Office and Office of Research and Development. Great Lakes connecting channels include Areas of Concern designated under the Great Lakes Water Quality Agreement due to legacy contamination and are the focus of ongoing remediation and restoration efforts. The NCCA probabilistic survey design allows estimates of condition based on water, benthic, and sediment quality. These condition estimates offer the opportunity to understand current conditions across the connecting channels and can aid in the design of restoration efforts and assessment of restoration success. To assess sediment quality, NCCA uses an index based on sediment chemistry and sediment toxicity. We compared population estimates to targeted sampling of sediment in AOCs in the HEC and SMR to better understand how the NCCA probabilistic design captures the range of conditions present in each system. For example, preliminary results from the 2014 HEC survey suggest that sediment quality was good in 48% of the survey area and that only 2% of the area was in poor condition. Targeted sampling of sediment chemistry in depositional areas of the Detroit River AOC showed that poor sediment conditions were underestimated in the 2014 survey. The area with poor sediment quality conditions was approximately the same as the error of the population estimates. An increase in point density or intensification in depositional areas would allow the assessment to capture fine-scale variability in the population estimate, but increase cost and effort. At the system-wide scale of the assessment, which included the navigational channel, the sediment quality conditions were fair to good. Sediment chemistry and toxicity data collected in 2015/2016 in HEC and SMR will be compared to respective targeted sampling efforts. Our findings highlight limitations of probabilistic sampling but also show the importance of system-wide studies for a holistic perspective of each connecting channel system.

468 A multi-disciplinary approach to monitoring the effectiveness of remediation and restoration at Onondaga Lake: Results-to-date

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Onondaga Lake in Syracuse, NY has been impacted by a long history of municipal and industrial discharges. Since 2004, the 12 km^2 lake has experienced significant improvements in water quality following upgrades to the Metropolitan Sewage Treatment Plant that reduced phosphorus and ammonia concentrations in the lake. In addition, Honeywell is completing the Onondaga Lake Superfund Site remedy in accordance with the Consent Decree. Key components include the dredging and capping of contaminated sediment, completed in 2016, and since 2011 the annual addition of nitrate to the hypolimnion to limit methylmercury release from profundal sediment. Habitat restoration including wetland creation and substrate improvement in the littoral zone was implemented in conjunction with remediation. To assess the effectiveness of remediation and restoration, Honeywell is developing a multi-disciplinary approach under the purview of NYSDEC and EPA. Chemical monitoring includes contaminant concentrations in cap material, sediment, water, and tissue (zooplankton, prey fish, sport fish). Innovative chemical measures include carbon and nitrogen stable isotopes to ascertain exposure pathways, and microbead placement in profundal sediment to enable monitoring of sediment accumulation associated with natural recovery. Biological monitoring includes fish size, age, and growth rate; fish and benthic macroinvertebrate community surveys; and wetland, upland, and in-lake vegetation. Results to date indicate declining mercury concentrations in water, zooplankton, and fish since baseline monitoring began in 2008. In addition to Honeywell's monitoring program, Onondaga County's Ambient Monitoring Program (AMP), the Upstate Freshwater Institute, and local universities monitor various lake characteristics relevant to lake restoration. For example, the AMP has documented increased macrophyte coverage and fish diversity. The combined monitoring programs will assist in evaluating the effectiveness of remediation in achieving risk-based and restoration goals and understanding the large-scale factors that potentially impact contaminant concentrations. Such factors include food web dynamics (e.g., occurrence of zebra mussel, quagga mussel, alewives, and large Daphnia), year-to-year variability in precipitation and temperature (that can impact nutrient and mercury cycling), changing water levels (controlled by dam and climate), and regional background Hg concentrations in fish.

469 Meeting Restoration Goals Following Sediment Remediation - An Analysis of Great Lakes Areas of Concern

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Background/Objectives The Great Lakes Restoration Initiative (GLRI) funding is responsible for a slew of restoration projects throughout the Great Lakes. This funding has been a crucial in restoring Great Lakes habitat. Although a large portion of the funding goes towards habitat restoration, there is still a need for evaluating the success of your restoration work in order to inform future/potential projects. We can measure the impacts of these restoration projects by looking at the Beneficial Use Impairments (BUIs) for the that AOC. For example, the Degradation of Benthos BUI and the Fish Consumption BUI are great indicators for the success of a project. The Great Lakes Legacy Act (GLLA) funding is another source of federal funding primarily used for sediment remediation. Before habitat can be restored, the sediment needs to be remediated – that's where the GLLA comes in. Approach/Activities Restoration Effectiveness – What is it? Why is it so important? What does the information tell us about the ways we conduct sediment remediation and habitat restoration? How do you measure it? (One way is by tracking the progress of your BUIs). When designing a restoration effectiveness project, what needs to be considered? Establishing an effective monitoring strategy is very important to the success of a project. Sampling methods need to be strategic and effective. Do you have realistic and achievable targets? If not, you may need to consider changing your criteria. What are some of the limitations that need to be considered when developing and effective monitoring strategy? How should your data be analyzed? Which methods or tools should be used for the analysis of your results? What tools are out there (innovative and effective tools)? Comparisons across sites are also very helpful for determining the validity of your results. Results/Lessons Learned There are many things to be considered before engaging in restoration effectiveness. For example: how long should you wait after remediation? Do you need technical assistance from federal agencies? (EPA ORD, USACE, USFWS) Does the data inform potential short comings of sediment remediation or habitat restoration? Is there anything that we can change to have more successful restoration? These questions and more will be answered during this session.

Current and Future Challenges in Sediment Toxicity Testing for Environmental Risk Assessment

470 Evaluation of the functional equivalency of negative and solvent control treatments from spiked-sediment toxicity studies

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In the United States, sediment toxicity testing is a conditional registration requirement for pesticides with Kd ≥ 50, Log Kow ≥ 3, or Koc values ≥ 1000. The hydrophobicity of these compounds often necessitates the use of solvents to ensure accurate dosing of spiked-sediment studies at target concentrations. Notably, for sediment toxicity tests a volatile solvent (*e.g.* acetone) is generally used as a transient carrier. Due to low water solubility, test material is fully dissolved in a volatile solvent, often acetone, to create a concentrated stock(s). A measured aliquot of the required stock is then evenly distributed to a sand substrate, after which the solvent is allowed to evaporate to dryness. This spiking process should result in negligible solvent exposure to organisms, and results demonstrate that test organism performance is comparable between negative and solvent control treatments. As such, the negative and solvent control treatments are functionally equivalent. In December 2016, the USEPA Office of Chemical Safety and Pollution Prevention (OCSPP) released a number of final ecotoxicity test guidelines, including methods for both the subchronic freshwater (OCSPP 850.1735) and marine (850.1740) sediment toxicity test guidelines. These methods provide an option for conducting experiments with only a solvent control, and no negative control treatment. To adopt this testing strategy, it must be demonstrated that there is functional equivalency between the negative and solvent control treatments. The 850.1735 and 850.1740 test guidelines describe specific factors that should be considered for evaluating functional equivalency including: A) Concentration of solvent in the test sediment after evaporation (e.g., analytically determined), B) Levels of solvent that are known to affect organism health, C) The potential for impurities in the solvent and their potential impact on organism health and; D) Historical organism performance of solvent vs. negative control. Each of the aforementioned factors will be addressed using data either generated or compiled by the CropLife America Ecotoxicology Working Group Sediment Subteam. The results of this analysis support the position that elimination of the negative control treatment requirement will not impact the robustness or interpretability of spiked-sediment toxicity test data as there is no functional difference between negative control and solvent control sediments.

471 Establishing equilibration of chemical concentration in pore water and sediments prior to conducting sediment toxicity tests

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In sediment tests sufficient time is needed for a spiked chemical to equilibrate within the test system prior to exposing test organisms. Shorter equilibration times may be needed for chemicals that degrade more rapidly in sediment while longer periods are needed for those that are more persistent. For organic chemicals, it is recommended to age sediment at least one month before starting a test. For chemicals with a high log Kow (e.g., >6), a period of two months or longer may be necessary, and for metals, a shorter time frame of 1 to 2 weeks may be sufficient. OECD 218 recommends a period of 24 – 48 hours of equilibration prior to exposure; however, ASTM E1706-05 states that this short time period may not be long enough for sediments to equilibrate with the spiked chemicals. The USEPA recommendation is that periodic monitoring of chemical concentration in the pore water during the sediment aging process should be used as a means to assess the equilibration of the spiked sediments. One technique referenced by USEPA suggests that spiked sediment be aged under refrigerated conditions in the dark for a month prior to use in the test and that samples of the aged sediment be analysed on a weekly basis to establish equilibrium in the sediment and pore water. While this technique shows equilibrium under stored conditions, it does not necessarily reflect test conditions. OCSPP 850.1000 suggests that flow-through systems may need to operate for a set period of time to obtain constant and representative concentrations. Another technique for determining equilibrium under testing conditions has been demonstrated by spiking the test chemicals into either natural or artificial sediments, mixing at least overnight to achieve homogeneity, adding the spiked sediment into the test beakers and then adding water in the test system. The pore water is analysed after typically 2, 7, 10, 14, 21 and 28 days in the test system and the analytical results are examined for signs of equilibrium (when two intervals do not differ significantly) or significant loss (at or below the limit of quantitation). These results are used to choose an appropriate equilibration time for the definitive test. The results of this type of analysis for a variety of chemical types support the position that equilibration of a test chemical can be established within the test system instead of under storage conditions.

472 Expanding the Number of Freshwater Invertebrates used in Acute Toxicity Tests for the Registration of Chemicals

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There has been an increased interest in expanding the number of freshwater invertebrate species used for acute toxicity tests for the registration of chemicals. Tests for these species have followed guidelines based on the acute daphnia test that represents a water only exposure for 48 hours. Some tests have been extended to 96 hours. The battery of test we have conducted include the larval stage of freshwater insects (mayfly - *Neocloeon triangulifer*, caddisfly - *Chimarra atterima*, stonefly - *Soyedina carolinensis*), chironomids (*C. riparius and C. dilutus*), crayfish (*Procambarus clarkii*), freshwater amphipods (*Gammarus pseudolimnaeus*, *Hyalella azteca*), lumbriculus (*Lumbriculus variegatus*), and the daphnids (*D. pulex and Ceriodaphnia dubia*). Protocols for many of these species have often cited the daphnia guideline for acute testing. However, our experience in testing to date has determined that there are species specific ecological requirements and challenges (e.g. sediment/substrate requirements, cannibalism, feeding during testing) that are not being accounted for in the daphnia guideline. A need for species specific guidelines appears to be in order. Our recommendation is that the invertebrate guidelines should be revised in order to address the diversity of organisms being included in risk assessments.

473 Method Improvement Through the Examination of Growth and Survival Differences Between Fed and Unfed Nymphs of Hexagenia spp. in Various Sediments

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Hexagenia spp. is an ecologically important burrowing mayfly that when used sediment toxicity assessments provides an understanding of the potential toxicity to an organism with very different physiology and ecological niche than the standard test species *Chironomus dilutus* and *Hyalella azteca*. *Hexagenia* spp. have been routinely incorporated into Ontario Ministry of the Environment and Climate Change (MOECC) sediment quality assessments in the province of Ontario, Canada for almost 25 years. In addition, *Hexagenia* spp.is incorporated into Environment Canada's (EC) BEAST (BEnthic Assessment of SedimenT) protocol and has been in use for over 20 years. There is a significant difference between these two methodologies however. The EC test protocol requires that the organisms be fed over the duration of the test while the MOECC method does not. In general *Hexagenia* spp. has been shown to be intermediate in sensitivity, between the common sediment toxicity test species *C. dilutus* (least sensitive) and *H. azteca* (most sensitive). However, of 36 field collected contaminated sediments in which all three species were tested in our laboratory, where significant impairment was seen in at least one species, *Hexagenia* spp. was ranked as the most sensitive species in 28% of the sediments. While studies have shown that *Hexagenia* spp. nymphs have to increase their feeding rate effort in low organic carbon (OC) sediments to meet their dietary needs, in our studies with field collected sediments there was high variability in the relationship between OC in control and reference sediments and *Hexagenia* spp. wet weight at test termination ($R^2 = 0.14$, $n = 43$) however the relationship was significant (paired t-test $p = 0.0005$). To assess the impact of feeding on the survival and growth of *Hexagenia* spp. in the 21-day sediment toxicity test, *Hexagenia* spp. were exposed to four sediments with varying OC levels. Half of the test replicates were fed weekly, and half were unfed. Feeding the *Hexagenia* spp. significantly affected the growth of the organisms while survival rates were less impacted. The OC content of the sediments also influenced the survival and growth rates of the organisms, but not to the same extent as feeding. The MOECCHexageniaspp. 21-day freshwater sediment method uncertainty and the implications of feeding on the interpretation of study results and application in risk assessment will be discussed.

474 A fugacity approach to a benthic invertebrate probabilistic risk assessment of cyclic volatile methyl siloxanes

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This study presents a risk assessment method which combines the thermodynamic principle of fugacity with a probabilistic analysis of field monitoring data and results from chronic laboratory benthic toxicity studies on the cyclic volatile methylsiloxanes (cVMS) materials; these compounds are octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5), and dodecamethylcyclohexasiloxane (D6). Benthic organisms are exposed to sediment-bound chemicals and data frequently exist for both sediment and biota matrices. A key risk assessment challenge is how to best compare divergent field data to toxicity trigger levels and check for consistency among the available field data. In this work, we applied a fugacity approach to assess the probability of risk to benthic organisms using different matrices such as sediment and biota in comparison to toxicity benchmarks. A probability risk assessment method was applied to D4, D5, and D6 using field sediment and biota data from Norway, Canada, USA, Japan, and numerous European countries, compared to chronic benthic laboratory toxicity study no-observed effect concentrations (NOECs). Sediment and invertebrate biota exposure data for D4/D5/D6 were individually sorted and the 95th centile exposure levels determined on a fugacity basis for both matrices. These cVMS exposure fugacities were then compared to interpolated 5th centile benthic sediment NOEC fugacity levels, calculated from a sorted distribution of chronic D4/D5/D6 toxicologic assays per OECD guidelines using a variety of standard benthic species. No overlap was noted for D4 and D5 95th centile sediment and biota fugacity levels and their respective 5th centile benthic organism NOEC values. For the D6 cVMS material, there was a small level of overlap at the exposure 95th centile sediment fugacity and the 5th centile benthic organism NOEC fugacity value; the sediment fugacities indicate that a small, quantifiable risk (~0.3%) exists for benthic species. In contrast, this small D6 risk potential was not replicated with field invertebrate biota fugacity data when comparing the exposure 95th centile biota fugacity and the 5th centile benthic organism NOEC fugacity value. The reasons for this contrast in D6 benthic risk assessment conclusions and other cVMS results will be discussed.

475 Challenges in interpreting sediment toxicity test and analytical results for development of cleanup benchmarks for coal tar contamianted sediment

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Coal tar contaminated sediment is a widespread problem in streams and estuaries adjacent to former manufactured gas plants (MGP) and wood treatment facilities. Measuring concentrations of polycyclic aromatic hydrocarbon (PAH) in sediment or pore water and conducting sediment toxicity tests with benthic organisms have been the fundamental approaches to ecological risk assessment and development of benchmarks to clean up contaminated sediment. Theoretically, the cleanup benchmark is directly related to the question of what an acceptable level of contaminant for an aquatic system can be. Practically, the benchmark value determined can significantly affect delineation of the extent of contamination for remediation and subsequently the project cost. Researchers and project managers face significant challenges in the process of developing such benchmarks. These challenges can originate from different methodologies used for sediment toxicity tests and chemical analyses; furthermore, sophisticated statistical analyses used to interpret results may lead to additional debates. To provide assistance for understanding the challenges and finding solutions, we will present results obtained from laboratory toxicity tests in which both benthic and water column organisms, particularly fathead minnow, were exposed to coal tar contaminated sediment. The potential significant effect of analytical uncertainties associated with PAH analyses on developing a cleanup benchmark will also be discussed. Based on the results, we believe that interpretation of toxicity test results for a coal tar contaminated sediment site must consider factors, such as representativeness of samples for toxicity testing, analytical uncertainties, presence of multiple contaminants, and fate and transport of coal tars. A few recommendations for sediment assessment and decision making include: 1) add sediment toxicity testing with water column organism to routine benthic sediment toxicity tests; 2) perform a dilution series of sediment toxicity test for development of a cleanup benchmark; and 3) consider additional data quality control in analyzing sediment samples for PAHs.

476 Comparison of acute and chronic toxicity test endpoints with benthic infaunal community measures

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Sediment toxicity is usually assessed by conducting laboratory bioassays on thoroughly homogenized, field collected, sediment samples. Typically these bioassays measure survival in a sensitive species following short term exposures (e.g., 10-days); longer term, chronic tests measuring sublethal responses (e.g., growth and reproduction) are not routinely used. While it is generally held that these bioassays provide a conservative assessment of the the potential for environmental impact, there are few studies comparing results of laboratory sediment bioassays to actual measures of benthic community health in exposed field populations. To establish the relative efficacy of lab based bioassays (i.e., both acute and chronic) in predicting potential impacts in exposed field populations, a multi-year, laboratory to field comparison study was conducted. Lab bioassays included standard 10-day acute toxicity tests measuring survival in four species of estuarine/marine amphipods (e.g., *Eohaustorius estuarius*, *Ampelisca abdita*, *Rhepoxinius abronius*, and *Leptocheirus plumulosus*) and two longer-term, 28-day sublethal tests with a marine polychaete, *Neanthes arenaceodentata* (survival and growth) and the amphipod *L. plumulosus* (survival, growth, and reproduction). A highly contaminated (e.g., metals, pesticides, and PAHs) and toxic sediment was mixed with a cleaner sediment of similar grainsize to produce a series of diluted contaminated sediment treatments (e.g., 0,6,12,25, and 50% by volume). Sediment treatments were placed in containers and deployed in the field. At specified intervals over a three year period these containers were retrieved from the field and analyzed for sediment chemistry , infaunal community composition and toxicity (acute and chronic endpoints). Laboratory based toxicity endpoints were then compared to measures of benthic community health in ecposed infaunal communities recovered from the field to evaluate the ability of the toxicity tests to accurately predict observed impacts on the in situ community. Results of these comparisons indicate that laboratory based tests provide highly conservative estimates of potential bethic community impacts with both acute and chronic test detecting effects at lower treatment levels than were detected in exposed field populations using traditional measures of community response.

477 Sediment Toxicity of Estuarine Protected Areas in Southeast Brazil

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Estuaries are important ecosystems providing goods and services that are both economically and ecologically relevant. The establishment of Marine Protected Areas (MPAs) aim to protect its habitats, biodiversity and ecological processes as a conservation instrument. Many MPAs are located close to contamination sources including industrial areas, harbors and marinas, agricultural zones, urban areas and sewage outfalls. The release of chemicals in the environment results in sediment contamination posing risk to benthic communities affecting thus its ecological functioning.In this study we assessedthe sediment quality of estuaries situated in two MPA, the Center and South Coast Marine Protection Area (APAMLC and APAMLS)in São Paulo State, Brazil: Rio Verde (RV); Rio Una do Prelado (BU); Rio Guaraú (GU); Rio Preto (RP) and Rio Itanhaem (RI). The benthic community was evaluated by ecological descriptors (richness, diversity and evenness) and biotic index (AMBI) for estimate the ecological quality status. Data were integrated by Principal Component Analysis (PCA). Results revealed moderate to low levels of contamination, with higher values detected at sites influenced by domestic effluent discharges (RP and RI). Sediments were toxic and effects related to confounding factors were also observed. Benthic descriptors, AMBI and PCA results corroborated these findings and showed a gradient of environmental quality that ranged from better conditions (RV, BU and GU) to poor (RP and RI). Based in these results we concluded that benthic environments in the estuaries of APAMLC present a good ecological status compared to APAMLS, where contaminant concentrations were linked to biological effects. Also the MPA conservation effectiveness is not assured once these areas are affected by pollution. Funding source: São Paulo Research Foundation (FAPESP).

Multiple Stressors II – Assessing Contaminant Effects in Ecosystems with Multiple Stressors

478 Evaluating potential acute and chronic effects of pesticide mixtures in streams from the Midwest, Southeast, and Pacific Northwest regions (USA)

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To assess the distribution and potential aquatic toxicity of pesticide mixtures in streams at the regional scale, the U.S. Geological Survey National Water Quality Assessment (NAWQA) Project measured dissolved concentrations of 115 pesticides and 111 pesticide degradates in weekly water samples from 77-100 streams in each of 3 U.S. regions (the Midwest, Southeast, and Pacific Northwest) over 10-14 weeks. Mixture complexity was related to land use and region. For urban streams, more pesticides were detected in the Southeast, the Midwest, or Oregon (median of 14-25 compounds per sample) than in Washington State (median of 3 compounds per sample). For agricultural streams, more pesticides were detected in the Midwest and Oregon (median of 27-33 compounds per sample), where cropland was prevalent, than in the Southeast Piedmont ecoregion or Washington State (median of 6-7 compounds per sample), where agricultural lands were largely pasture. The Pesticide Toxicity Index (PTI, an additive model for assessing potential toxicity of mixtures) for benthic invertebrates was higher in urban than agricultural streams in the Midwest and Southeast, but higher in agricultural than urban streams in Oregon and Washington. Aquatic-life benchmarks and the PTI were combined in an evaluation of potential acute and chronic toxicity to invertebrates, including the role of mixtures. Acute toxicity was predicted if pesticides exceeded acute benchmarks, or if PTI >1, in any sample. Chronic toxicity (evaluated by site, not by sample) was predicted for a pesticide at a site if its maximum 21-day moving average concentration exceeded either chronic benchmarks or a PTI-toxic unit threshold of 0.1. Using the Midwest as an example, the invertebrate PTI was >1 in 2% of samples and at 11% of sites, with a single insecticide always dominating the PTI. This indicates that individual insecticides dominated potential acute toxicity in these discrete weekly samples, although this design clearly underestimates acute exposures. Invertebrate chronic-effect thresholds were exceeded in 55% of streams for one or more pesticides, and in 28% of streams for multiple (2-4) pesticides. In 12% of streams, chronic exposures were punctuated by an acutely toxic (lethal) insecticide pulse—usually imidacloprid or an organophosphate insecticide. The role of mixtures in chronic, sublethal toxicity is unknown, but concurrent exposure to multiple pesticides at chronic-effect levels was common in Midwest streams.

479 Can DGT predict metal mixture toxicity to two Antarctic marine microalgae in laboratory bioassays?

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Contaminants predominantly occur in mixtures, posing a challenge to environmental management which is predominately based on single-contaminant toxicity. Chemical interactions of the contaminants and non-specific biological responses to these mixtures may result in effects that differ from the sum of the toxicity of individual components. These differences can be classed as antagonism (less toxicity than expected from the sum of the individual contaminants in the mixture), non-interaction (toxicity equal to that expected from the sum of individual contaminants), and synergism (more toxicity than expected from the sum of individual contaminants). Diffusive Gradients in Thin-films (DGT) have been established as a robust method of analysing the biologically-available contaminants in situ and are well-positioned to predict the toxicity of contaminant mixtures. This study explores the use of DGT

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(with a Chelex-100 resin) to predict the toxicity of Cd, Cu, Ni, Pb, and Zn, in mixtures to two common Antarctic marine microalgae: *Phaeocystis antarctica* and *Cryothecomonas armigera*. DGT devices were optimised for use in Antarctic conditions by determining diffusion coefficients for each metal and quantifying the metal-absorbing capacity of the resin in marine waters at 1°C. Preferential binding of metals to the resin was observed prior to reaching the resin's capacity, with Cd, followed by Pb, and Zn, outcompeting Cu and Ni, which retained linearity of uptake until capacity. Non-interactive and synergistic toxicity was observed in the two algal species in response to increasing multiples of the environmental mixture (where the ratio of metals were based on reported concentrations at a historically contaminated Antarctic marine bay). Whereas, non-interactive toxicity was observed in response to an equitoxic mixture (five metals at their EC10 concentrations), as determined by Independent Action and Concentration Addition modelling.

480 Effects of Temperature and Bifenthrin on the Endocrinology of Juvenile Chinook Salmon (Oncorhynchus tshawytscha)

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The San Francisco Bay-Delta is experiencing seasonally warmer waters and salt water intrusion into historically freshwater ecosystems due to climate change. Juvenile endangered Chinook salmon (*Oncorhynchus tshawytscha*) inhabit affected waterways from juvenile development through the smoltification (or saltwater acclimation) process. Runoff events cause surface water contamination with pyrethroid pesticides, in particular bifenthrin, in Bay-Delta waterways. Thus, juvenile fish may experience increased seasonal temperatures during runoff events, and the effect of these warmer temperatures and bifenthrin exposure on pre-smolt Chinook are unknown. To increase our understanding of the potential interaction between temperature and bifenthrin exposure on early salmonid development, juvenile alevin and fry were reared within 11^0C, 16.4^0C and 19^0C fresh water for 11 days and two weeks, respectively, and exposed to 0, 0.15, and 1.5 µg/L bifenthrin for the final 96 hours of rearing. Predatory avoidance behaviors were recorded after bifenthrin exposure using a Y-Maze assay and analyzed using behavioral tracking software (EthoVision XT). Estradiol-17B (E2), testosterone, cortisol, triiodothyronine (T_3), thyroxine (T_4) levels were measured in whole-fish homogenates using hormone-specific ELISAs. Brain gonadotropin-releasing hormone receptor (GnRH2), growth hormone receptor (GHR1), and dopamine receptor (DR2A) mRNA levels were measured using qPCR. Preliminary results show significantly decreased survival and lower condition factors (indicator of fish health) in both juvenile stages with increasing temperature. Fry exposed to 1.5 µg/L bifenthrin had lower condition factors for all temperature exposures, indicating overall reduction in health. Brain mRNA transcripts increase with increasing temperature in fry, but 1.5ug/L bifenthrin caused a reduction in GHR1 and GnRH2 in fish treated at 19^0C. These trends were not seen in alevin, thus suggesting stage-dependent differences in temperature-mediated bifenthrin toxicity. This research highlights the different impacts of temperature and bifenthrin to each juvenile stage due to targeted developmental time points. This material is based upon work supported by the Delta Stewardship Council Delta Science Program.

481 Incorporating Spatially Explicit Population Models as the Endpoint of a Bayesian Network- Relative Risk Model

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Endpoints in Environmental Risk Assessment (ERA) are critical because they dictate the relevance of the assessment to stakeholders, and drive interpretation of risk in policy. Population-level endpoints may introduce less uncertainty than individual-level endpoints because the latter are often extrapolated to protect populations through the application of safety factors. Population-level endpoints also provide greater ecological relevance than individual-level endpoints in ERAs, because the population is often the level at which environmental management decisions are made. However, many population-level risk assessment endpoints do not reflect the spatial and temporal heterogeneity of the populations they represent, and thus preclude an understanding of how population viability is affected by toxicants on a regional scale. This research aims to develop spatially-explicit matrix metapopulation models for incorporation into a new Bayesian Network-Relative Risk Model (BN-RRM) using an adverse outcome pathway (AOP) framework to probabilistically relate sub-lethal and lethal effects of toxicants and environmental stressors. As a case study for developing this model framework, we are examining the impacts of organophosphate (OP) insecticides on Endangered Species Act-listed Chinook (*Oncorhynchus tshawytscha*) salmon populations using site-specific data from the Lower Skagit, Nooksack, Cedar, and Yakima River watersheds in Washington State. Watershed-specific Chinook salmon population models that reflect local metapopulation dynamics and population productivity will be developed and incorporated as spatially and temporally heterogeneous endpoints. This probabilistic framework integrates local aquatic toxicant concentrations, dose-response equations, and regional environmental conditions into age-specific changes in survival, which drive probabilistic population size outcomes that reflect the available data for regional toxicant concentrations and environmental conditions. Current model results indicate that Chinook populations are equally sensitive to toxicological and environmental effects, and differences in the importance of these effects are driven by differences in regional toxicant concentrations, dissolved oxygen, and temperature.

482 Assessment of Ultraviolet Light-Enhanced Toxicity of Oil to Early Life Stages of Estuarine Species

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Photo-enhanced toxicity is an important consideration in oil spill response because the spatial and temporal extent of negative effects to aquatic organisms may be underestimated if based on standard laboratory bioassays with fluorescent lighting. Many early life stages, which are often translucent, congregate at the surface, or in the upper mixing layer of coastal waters. This makes them prone to significant ultraviolet (UV) light exposure, combined with physical interaction with thin sheens of oil at the surface. To evaluate the extent and mechanisms of UV-enhanced toxicity, this project examined both water-accommodated fractions of Louisiana Sweet Crude (LSC) oil and thin sheens to early life stages of fish and crustaceans (sheepshead minnow, *Cyprinodon variegatus*, and grass shrimp, *Palaemonetes pugio*). Potential pathways for interaction between UV light and the chemical constituents of oil were experimentally manipulated, including direct physical interaction of the organisms with the thin oil sheen on the water surface, photomodification of the chemical constituents of oil into more toxic forms, and photoactivation of chemical constituents of the oil within the organism to more toxic forms. Effects of short-term exposures on mortality and subsequent fish and shrimp growth and development were assessed. Chemical profiles of LSC oil preparations with and without UV exposure were characterized. Results will be presented with an emphasis on the utility of these data in oil spill response and environmental assessment.

483 Bridging the gap between multiple stressor data and reality: A case study with Daphnia population dynamics and individual-based modeling

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A key element of successfully managing chemical contaminants is the estimation of effects on ecological receptors. The complexity and variability of real ecosystems presents a challenge to effect estimation. The essence of effect estimation for risk assessment—a combination of toxicity tests and mathematical models, generally falls short in addressing the influence of multiple stressors. Improvements in the current paradigm should perhaps concurrently focus on both toxicity test design and mathematical methods. In fact, recent efforts to improve toxicity test design have been geared towards increasing relevance and mechanistic understanding. These steps not only meet data needs of detailed models, but allow for more complex model construction and validation. As a demonstrative case study, we developed and implemented a dynamic energy budget individual-based model (DEB-IBM) for *Daphnia magna* populations to predict population-level effects of the fungicide pyraclostrobin while also conducting toxicity experiments in which *Daphnia* populations were exposed to pulses of pyraclostrobin. Simulation results indicated that standard acute toxicity test data from unfed neonates were not successful in predicting observed *D. magna* population dynamics after exposure to pyraclostrobin. We hypothesized that the ‘secondary’ stressor of density-dependent food limitation may cause the mismatched pyraclostrobin sensitivity in low food versus high food scenarios. We next exposed individual neonate daphnids to pyraclostrobin over a range of food levels. As hypothesized and observed, increased food led to increased resistance to pyraclostrobin. When this observation was quantified and incorporated into the DEB-IBM, the model was better able to capture decline and recovery dynamics of daphnid populations. Additionally, model output suggests that timing and exposure duration of secondary stressors may drive impacts on population resilience and should perhaps be explored explicitly in population-level experimental designs. In summary, this case study demonstrates that adjusting experimental design to improve multiple stressor model calibration and validation is not unreasonable. Further, adjustments to multiple stressor experimental design and model development can co-occur and will likely lead to improved effect estimation and higher confidence in risk assessments in actual ecosystems.

484 Improved Ecotoxicological Impact Assessment Models for Chemical Mixture Effects Using a Nonlinear Toxic Equivalence Method

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Forecasting the environmental impacts of novel materials allows technology developers, program managers, and other stakeholders to make informed decisions concerning the potential environmental, fiscal and regulatory costs of those materials throughout their life cycle. Financial liabilities incurred by the Army, even after execution of required environmental assessments, serve to exemplify both the potential threat involved in the use of inaccurate forecasting models and the risks to the development and sustainability of new military materials. To address this problem, the Army has begun to use Life Cycle Assessment (LCA) as a framework in which to evaluate ecotoxicological impacts throughout the material acquisition and decommission process. However, because the functional properties of many military materials inherently rely on multicomponent mixtures, and while the congeners are often themselves individually toxic, the mixture itself may exhibit a combined toxicity not captured by existing impact assessment methods. This is because existing methods model toxic effects entirely in terms of the individual substances only. To fill this critical knowledge gap, we have developed ecotoxicological models for mixtures including multiple chemicals and substances. To model impacts on the species sensitivity distribution from these mixtures, we developed a method that extends the traditional toxic equivalence (TEQ) approach used extensively for polychlorinated biphenyl (PCB) toxicity, which underlies the equivalency factors (EFs) used throughout many life cycle impact assessment (LCIA) methods. We exemplify this new method using data from environmentally relevant multi-component mixtures. Finally, we evaluate the data requirements of the model by conducting a sensitivity analysis that identifies critical parameters requiring high precision (low uncertainty) of the input data. Our models improved ecotoxicological impact characterization by accounting for biological effects over the entirety of the (nonlinear) dose-response, which stands in contrast to existing methods that rely tenuously on assumptions of dose-response linearity.

485 How to Integrate Adverse Outcome Pathways into a Probabilistic Regional Ecological Risk Assessment

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A challenge in environmental toxicology and risk assessment has been the extrapolation from the molecular interactions within an organism to landscape scale effects on population dynamics and community structure. The l framework is derived from the Bayesian network relative risk model that has been used at a variety of scales, from watersheds to continental regions. The first step is the transformation of the required adverse oucome pathways into the basics of an Bayesian network. Our model is based on the AOP for organophosphates proposed by Ankely et al 2010 and Russom et al. (2014). The basic structure is similar but with outputs nessary to inform a population model to determine effects. The next step is that the AOP influence diagram is placed into an ecological risk assessment model based on the BN-RRM process as demonstrated by Landis and colleagues. The sources of the stressors are identified, the stressor exposures quantified and the exposure-response relationships are represented as the conditional probability tables in a Bayesian network. Other ecological factors such as water temperature, habitat quality and other features are represented by similar pathways. The results of these exposures are calculated for each of the endpoints. One of the advantages of using the Bayesian network approach is that other quantitative models or expert solicitation can be used to describe the interactions. Two examples will be used to illustrate the approach. The first is a simplified description of the effects of organophosphates in the Nooksack River, part of the Puget Sound watershed. The second example is based upon the Pacfic herring run at Cherry Point and incorporates PAHs and the projected alterations in water quality and habitat due to climate change. Finally we will demonstrate how this framework can be used to integrate ecological risk assesment with human health and well being.

Environmental Chemistry – Part 2

486 Potential dietary exposure to PFASs of South Carolina hunters from recreationally harvested alligator meat

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Exposure to perfluoroalkyl substances (PFASs) has been linked to many harmful health effects including reproductive disorders, developmental delays, and altered liver and kidney function. Most human exposure to environmental contaminants, including PFASs, occurs through consumption of contaminated food or drinking water. This study determined PFAS concentrations in tail meat samples collected from recreationally harvested American alligators (*Alligator mississippiensis*) in South Carolina to assess potential dietary exposure of hunters and their families to PFASs. In addition, consumption patterns were investigated using intercept surveys of 23 hunters at a wild game meat processor. Perfluorooctane sulfonic acid (PFOS) was the dominant PFAS found in samples (median 6.73 ng/g). With alligators harvested in the Middle Coastal hunt unit having significantly higher PFOS concentrations than other hunt units in the study (median 16.0 ng/g, $p = 0.0001$, $n=17$). Using the average consumption frequency, portion size, and median PFOS concentration in alligator meat from all hunt units found the daily dietary exposure to be 2.11 ng/kg body weight per day for an adult human. However, dietary PFOS exposure scenarios based on location of harvest suggested the highest daily exposure occurs with alligator meat from the Middle Coastal hunt unit in South Carolina. Current consumption patterns and PFAS concentrations do not exceed suggested exposure guidelines; however further studies on site-specific exposure and consumption by vulnerable populations need further research to protect human health.

487 Elevated Blood Lead Levels Among Children, Infants and Mothers in Kabwe, Zambia

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Lead (Pb) poisoning has always been a serious human health concern, and is one of the most common and best-recognized childhood diseases of toxic environmental origin. Lead poisoning is entirely preventable, yet it accounts for about 0.6% of the global burden of disease, with the highest burden in developing countries. Kabwe town, capital of the Zambian Central Province with a population of approximately 203,000, is among one of the most polluted places in the world. The town has a long history of leadzinc mining, which operated for nearly 100 years without any pollution laws regulating emissions from the mine, and addressing the potential dangers of Pb contamination. Despite closure of the mine, scavenging of metal scraps from the abandoned tailings, use of lead-laced soil to make bricks, dust emanating from the mine dumps, etc… have continued to serve as a source of metal pollution. This present study investigated blood lead levels (BLLs) in children and infant-mother pairs living around and far from the closed Pb-Zn mine in Kabwe. In total, 153 children's and 417 infants'-mothers' paired blood samples were collected. The system used the venous blood, and BLLs were measured using the Lead Care II analyzer System. The result showed the prevalence of Pb poisoning in the townships near to the mining area. 93% children and 77% infants BLLs exceeded the CDC recommended level of 5mg/dL. Furthermore, ten blood samples from infants exceeded the level that can cause encephalopathy and even death (100 µg/dL). A significant correlation between BLLs of paired mothers and infants was detected. This clearly demonstrates the ability of Pb to transfer via breastfeeding. We also compared BLLs from children recruited in 2014 by pure earth, and the result showed a decreased trend as age increased. In general, mothers and infants with high BLLs, children who play in the soil, and young men and women who artisanally mine in the area are most susceptible to Pb and, therefore, they are at high risk. Finally, it is recommended that periodic monitoring of blood Pb in infants and children, and educating the local population should be undertaken to control the Pb blood levels in the whole population.

488 Metals and PAH contamination of Cultus Lake, BC: Risks to federally listed populations of sockeye salmon and coastrange sculpin

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Cultus Lake, BC, Canada, is habitat for two federally listed species at risk: the endangered sockeye salmon, Cultus population (*Oncorhynchus nerka*) and the threatened Coastrange sculpin, Cultus population (*Cottus aleuticus*). The Cultus population of sockeye salmon collapsed post 1970 due to overexploitation in mixed-stock fisheries, and at current low abundances is further at risk from degradation of Cultus Lake.The Cultus population of Coastrange sculpin has evolved extremely rare traits, and its limited range makes it susceptible to changes within the lake, including those resulting from the decline of the sockeye salmon population. Within Cultus Lake, anthropogenic stressors include habitat destruction, invasive species, heavy recreational use, and pollution. Concentrations of metals and PAHs in waters and sediments of Cultus Lake can exceed water and sediment quality guidelines for the protection of aquatic life. With sediment cores, we are identifying sources of metals and PAHs to Cultus Lake, as well as tracking temporal changes in concentrations and fluxes, to understand past, present, and future risks to these fish populations from pollution. Principal component analysis grouped metals into three categories: (1) lithogenic (e.g., Ti, Al), in which the source is soil and bedrock in the lake's watershed, though delivery to the lake can be accelerated by development; (2) diagenetic (e.g., Mn, As), in which natural processes constantly redistribute metals to the redox boundary; and (3) pollution (Pb, Sn, Hg, Ag, Zn, Cd, Sb, Cu). Sources of "pollution" metals include gold mining (Hg only), coal combustion, smelting, leaded gasoline (Pb only), and the addition of copper sulfate to control swimmer's itch (Cu only). Sixteen priority PAHs were measured, nearly all exhibit a similar increase in concentration post 1850, and diagnostic ratios suggest a shift of predominant source from degradation of organic matter to coal combustion. With the exception of Hg, Sn, anthracene, and dibenz[a,h]anthracene, concentrations of metals and PAHs have been declining for at least the past three decades. It is unlikely that pollution contributed to the collapse of the Cultus population of sockeye salmon, but pollution can limit recovery of populations at low abundances. Research is warranted to investigate exposure and effects on early-life stages of individuals of Cultus populations of sockeye salmon and Coastrange sculpin (or surrogates).

489 Long-term Monitoring of Polychlorinated Biphenyls (PCB) in Brook Trout Muscle Tissue

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A waste treatment facility operating in central Alberta since 1987, is capable of safely processing polychlorinated biphenyls (PCBs) by incineration. Since 1985 (two years of baseline study before operations), annual analysis of PCBs in various media from the surrounding area (including snow, air, soil, vegetation, wildlife, groundwater, surface water, sediment, and fish tissue) has been the focus of a monitoring program required under the facility's operating approval. Chemical concentrations in fish tissue are studied each year to assess ecological and human health risk from processing activities. Muscle tissue samples taken from brook trout (*Salvelinus fontinalis*) stocked into nearby high-elevation

lakes are selected from fish of different ages to evaluate bioaccumulation of PCBs. Traditional aging methods are inaccurate because of conditions in the hatchery during rearing. Therefore, all brook trout stocked into the study lakes since 2012 have been implanted with coded wire tags that provide a definitive fish age. Brook trout PCB tissue concentrations have not increased based on limited numbers of fish analyzed, but several instances of higher PCB concentrations in older fish suggest bioaccumulation is possible, which warrants further investigation. Potential exposure pathways have been investigated in 2012 and 2014 using semi-permeable membrane devices and analysis of PCBs in aquatic macro-invertebrates. Results indicate that direct uptake from the water column may be limited, and that food web interactions may represent a potential exposure pathway for fish. Results are presented annually to regulators, stakeholders (local indigenous peoples, recreational users, and nearby residents) and consultants to identify potential environmental concerns and program recommendations. Current program goals include addressing limitations of small sample sizes, reconstructing life-histories to better quantify possible bioaccumulation of PCBs in fish tissue, and quantitatively comparing PCB congener profiles between age classes and reference fish. This study will outline the benefits, challenges, and limitations of using congener-specific PCB analysis of fish tissue for long-term risk assessment.

490 An assessment of elemental concentrations and radioactivity in biota during and shortly following active uranium mining in the Grand Canyon watershed

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The U.S. Geological Survey and its cooperators are currently engaged in biological monitoring of reference and mining sites in pre-, post- and active-uranium ore production stages in the Grand Canyon watershed (Arizona, USA). The present work describes the results of chemical (e.g., nickel, copper, zinc, arsenic, selenium, lead, thallium, uranium) and radiological analyses of mammals, vegetation, and other tissues collected at two mine sites. The Pinenut site was mined in the 1980's before being placed on standby in 1989; mining was resumed in 2013 and completed in 2015. Sampling of the site was performed in 2014-2015 during active mining. Pre-mining activities at Arizona 1 began in 1990 and completed in2008, with active mining occurring from 2009 to 2014. The 2014-2015 sampling at Arizona 1 was performed post-active mining, but prior to site reclamation. Contamination at Pinenut and Arizona 1 may represent a worst-case scenario for biotic exposure, with ore being brought to the surface and stored aboveground for transport, aeolian transport of contaminants related to the ore body and waste rock, active mine dewatering, and rain transport of contaminants into the mine detention ponds and off site. The chemical and radiological results for Pinenut and Arizona 1 will be compared to data obtained for animal and plant tissues at the Canyon Mine, a pre-mining site currently undergoing shaft sinking; increased uranium concentrations and gross alpha activity have been observed at these sites compared to Canyon Mine. Concentration results will be compared to literature-based toxicity thresholds and used to help inform on the ecological effects of uranium mining in the Grand Canyon watershed.

491 Evaluating Microbial Resilience to Inhibitor Exposure of Composite-Coated Encapsulated Bacterial Cultures in High Strength Wastewater

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Production of hydrogen gas from dark fermentation of organic waste has been studied extensively as a potential source of renewable energy. Nevertheless, several major hurdles to large-scale production and non-centralized application have yet to be resolved. One such barrier is the design of efficient, stable, and resilient cultures that are resistant to shock loadings of inhibitory agents. It is postulated that encapsulation will provide the benefit of microbial resilience and resistance to inhibition as a result of the high biomass levels present when encapsulated as well as the ability of the alginate gel to complex with cationic inhibitory species. Mixed cultures of anaerobic digester-derived hydrogen-producing bacteria were encapsulated in composite-coated calcium-alginate beads. Encapsulated bacterial cultures were then exposed to 24 hour shock loads of inhibitory agents – chloroform, ammonia, dichromate, and copper. After a 24 hour exposure period the cumulative hydrogen gas generated was compared to the gas production of inhibitor-free control cultures. The degree of inhibition for each inhibitor was compared to that for an equivalent concentration of suspended growth biomass. It was observed that for chloroform and dichromate, a significant decrease in inhibition was observed, with IC50 (the 50% inhibition concentration) values 2.68 and 1.89 times higher for the encapsulated cultures. For copper, no inhibition was observed over the inhibitor dose range (2 – 16 mg/L Cu) for the encapsulated species, whereas nearly complete inhibition was observed (> 90%) for the suspended cultures at > 5 mg/L Cu. For ammonia, only slight inhibition was observed at extremely high doses (6 – 15 g/L) for suspended cultures with decreased resilience observed for the encapsulated bacteria. Near complete recovery (> 95%) was observed for all inhibitors for both suspended and encapsulated cultures with the exception of encapsulated bacteria exposed to high doses of ammonia (> 10 g/L N). For these samples, significant degradation of the composite coating as well as the beads themselves was observed after the 24 hour exposure period.

492 Determination of Fungicides, Diamide and Neonicotinoid Insecticides in Water by Direct Injection Liquid Chromatography Tandem Mass Spectrometry

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An analytical method that uses liquid chromatography tandem mass spectrometry (LC-MS/MS) was developed for determination of 30 current-use pesticides in filtered water samples from streams and groundwater. This method expands upon an existing method that determines 225 pesticide compounds, also using LC-MS/MS, in order to evaluate potential for using the method on a more sensitive LC-MS/MS instrument. The 30 new pesticide compounds in the method include fungicides, diamide and neonicotinoid insecticides, and other pesticides that are widely used or recently registered for use in the United States. The method uses direct injection of a 20-µL water sample into the LC-MS/MS instrument; no sample preparation other than filtration is necessary. Samples are analyzed in electrospray ionization (ESI) positive mode using dynamic multiple reaction monitoring (MRM) conditions, with two MRM transitions for each compound. The LC and ESI parameters were optimized to select conditions that provided the highest sensitivity for the most compounds. Method detection levels (MDL) for the new pesticide compounds range from 1 to 9 ng/L, with exception of tribenuron-methyl with an MDL at 26 ng/L. This is equivalent or lower than the MDLs of other pesticide compounds determined with a less sensitive instrument, even with a reduction in injection volume from 100 to 20 µL (to reduce matrix interferences). The method was used in a USGS National Water-Quality Assessment project assessing stream quality in the Central California coastal region to assess method performance in variety of stream sample matrices in conditions expected in routine laboratory operation. Water samples were collected weekly from 85 sites sampled for 4-6 weeks during March-May 2017 and analyzed for pesticide compounds by LC-MS/MS (42 to 99 samples per week). Preliminary results indicate good performance of the method in terms of agreement in replicate concentrations (< 21% RPD), and maintenance of MDLs throughout the analytical batch. Some pesticide compounds were found in every weekly sample at sites located in agricultural areas in the Central Coast, with the fungicides boscalid and mycobutanil, the diamide insecticide chlorantraniliprole, and neonicotinoid insecticides clothianidin, imidacloprid, and thiamethoxam found most frequently. The direct injection LC-MS/MS method is suitable for quantitative analysis of new pesticide compounds at concentration levels relevant for environmental monitoring studies.

493 An effect of time trends of 64 pesticides concentration on the time-weighted average concentrations by passive samplers in surface water

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Monitoring of a broad spectrum of water contaminants is needed to protect sound aquatic ecosystem and human activities. Most of Japanese environmental monitoring of aquatic pollutants is performed by grab sampling method. The grab sampling data in base flow condition is generally assumed as monthly average concentration though it is "snapshot data". This concept is easy to understand, however regulation based on this data is dangerous. For example, strong rainfall events of short duration may cause rainfall- runoff and much higher concentration of nutrients, heavy metals, polyaromatic hydrocarbons and other pollutants can be detected. Moreover, it is more dangerous to human health and wildlives that some of regulated chemicals and non-regulated very harmful chemicals (unknown?) may be released to the environment intentionally or non-intentionally and the peaks can be lost. In order to solve these problems, new grab sampling method coupled with passive sampling is suggested by many scientists who researches passive sampling techniques. Meanwhile, no useful applications are proposed about how to use both grab sampling data and time-weighted average concentration data (TWA). In this study, 64 pesticides monitoring which combined grab sampling and passive sampling was performed during several months at some rivers to reveal an effect of time trends of the concentrations on TWA concentrations by passive samplers. These results indicated that passive sampling could detect comparable numbers of pesticides which were detected in grab samples. It also indicated that TWA concentrations by passive samplers were good agreement with those by grab samples within two times when there were no sharp peaks in the time trends. On the other hands, it was apparent that ratios of TWA by grab samples to TWA by passive sampling had a linear relationship to coefficient of variation of time trend data when ratios of TWA by grab samples were more than five times larger than TWA by passive samplers. This results suggested that sharp peak events such as runoff by rainfalls could be existed when one grab sampling data was five times larger than TWA concentration data by passive samplers.

WITHDRAWN

Existing and Emerging Contaminants in Changing Arctic Environments

494 Trends of legacy and emerging contaminants in ringed seals from the Canadian Arctic: When science and traditional knowledge meet

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The ringed seal (*Phoca hispida*) is the most abundant Arctic pinniped with a circumpolar distribution and has been a key biomonitoring animal for examining trends of persistent organic pollutants and mercury in the Arctic. This project began in 2004 under the Northern Contaminants Program (Indigenous and Northern Affairs Canada) and aimed to address the spatial and temporal trends of legacy and emerging contaminants in ringed seals across the Canadian Arctic. The project involved annual sampling in the Northwest Territories (Sachs Harbour), Nunavut (Resolute Bay and Arviat) and Labrador (Nain). All sampling has been done by local harvesters and coordinated by Hunters and Trappers Associations in each community. Spatial and temporal trends of polychlorinated biphenyls, flame retardants, perfluoroalkyl substances, polychlorinated naphthalenes and mercury will be discussed in this presentation. Traditional knowledge (Inuit Qaujimajatuqangit) and local engagement of each of the participating Inuit communities is key to understanding the ecology of seals and the Arctic environment. Thus, we initiated the idea of complementary community workshops on ringed seal health which started in Resolute Bay in 2016 and in Sachs Harbour in 2017. This educational component provides opportunities for Inuit elders to share their knowledge in seal ecology and traditional methods with students and researchers. Additionally, the workshops aim to increase the capacity of northern residents, with a focus on youth, to increase local understanding and engagement in ongoing scientific research on northern contaminants. Throughout the workshop process the identification of appropriate communication practices and the development of innovative methods of community engagement around contaminants in wildlife are being done through a series of surveys and discussions with workshop participants. Overall the goals of this complementary project are to raise awareness of contaminants in the North, help to support food choice decisions among consumers of traditional/country foods and build capacity in the North to contribute to addressing contaminants-related ecology and human health issues.

495 Dissolved organophosphate esters in Fram Strait deep waters and Canadian Arctic surface waters

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Recent studies have shown that organophosphate esters (OPEs) are capable of long-range transport to remote environments, but their transport mechanism and ultimate fate remain poorly understood. Few measurements of OPEs in remote seawater and lakes are available, with no measurements from deep ocean waters. To investigate the occurrence of dissolved OPEs in remote aquatic environments, polyethylene passive samplers (PEs) deployed at deep mooring stations in Fram Strait from June 2014 to July 2015, and PEs deployed at surface water sites in the Canadian Arctic during June to August of 2015 and 2016, were retrospectively analyzed by GC/MS for four halogenated OPEs and seven alkyl/aryl-substituted OPEs, as well as other organic flame retardants. In the Fram Strait, where seawater is exchanged between the Arctic and North Atlantic Oceans, OPEs were measured in PEs deployed along depth profiles from 200 to 2,500 meters. Σ_7OPE ranged from 80 pg/L to 500 pg/L, with the three chlorinated OPEs (TCEP, TCIPP, and TDCIPP) most abundant. Depth profiles were unexpectedly flat, possibly due to a high degree of vertical mixing and/or release of particle-bound compounds into the dissolved phase at depth. The presence of OPEs in deep North Atlantic waters suggests that these emerging contaminants have already become widespread in the Arctic Ocean. In Canadian Arctic surface waters, Σ_7OPE ranged from 370 pg/L in Barrow Strait to 4400 pg/L in East Lake on Cape Bounty, with three chlorinated OPEs making up >90% of total OPEs at each site. In contrast, total dissolved polybrominated diphenyl ethers were found at < 5 pg/L at all surface water sites, and at < 1 pg/L at all deep mooring sites, highlighting the importance of OPEs as ubiquitous dissolved organic contaminants with poorly understood impacts on remote aquatic environments.

496 Temporal Trends of Emerging Halogenated Organic Contaminants in Beluga Whales (Delphinapterus leucas) from the Western and Eastern Arctic

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Limited information regarding the contemporary and/or historical emissions exists for many anthropogenic chemicals. Because of their abundance, ecological significance, and that they are top trophic level animals, beluga whales make an ideal bio-indicator species for tracking the emissions of chemicals into the environment. This study examined the temporal trends of polyfluorinated chemicals (PFCs), polybrominated diphenyl ethers (PBDEs), and hexabromocyclododecane (HBCD) isomers in beluga whales from three populations from 1982 to 2013. Samples were obtained from traditional subsistence hunts for the three populations; the Eastern Beaufort Sea population samples were collected at Hendrickson Island (HI, NT), the Cumberland Sound population samples were sampled in Pangnirtung and the western Hudson Bay population samples were collected in Sanikiluaq (NU). This analysis showed considerably greater concentrations of $\sum_2$HBCD (sum of α- and β-isomers) than $\sum_6$BDEs (sum of BDE-47, -85, -99, -100, -153, and -154 congeners) at all locations. C_8-C_{12} Perflurocarboxylic acid ($\sum_5$PFCA) concentrations declined in animals from HI over the study period, while no trend was observed for animals from Pangnirtung. A linear increase in perfluorooctanesulfonic acid (PFOS) concentrations was observed in animals from HI between 1984 to 2000 and remained relatively stable thereafter until a notable increase in 2011 and subsequent decreases in 2012 and 2013. A similar temporal trend profile was observed for PFOS in animals from Pangnirtung. In 2013, concentrations of $\sum_5$PFCAs in animals from Sanikiluaq were significantly smaller than those of HI animals that same year, whereas perfluorooctanesulfonamide (PFOSA) concentrations in animals from Sanikiluaq were ~2x greater than those from HI. Confounding factors of size and age of animals sampled between these locations are believed to be key drivers of the differences observed in concentrations and trends. No correlation between PFOS and PFOSA concentrations was measured in any location.

497 Modeling the effects of climate change on the temporal trends of contaminants in marine, terrestrial and avian wildlife of the Canadian Arctic

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Legacy persistent organic pollutants (POPs) and mercury (total mercury; THg) continue to be reported at appreciable levels in arctic environmental media including in wildlife despite global initiatives to regulate these contaminants. Long-term trends of legacy POPs and mercury in arctic wildlife vary spatially, by biome and by species. Despite the attention given to climate-related issues in the Arctic, the environmental changes most relevant to temporal and spatial trends of contaminants in arctic wildlife have not been established. The current study focussed on compiling and establishing which climate-related variables significantly affect the trends of legacy POPs and THg in wildlife from around the Hudson Bay region. Under study were polar bears (*Ursus maritimus*) from southern and western Hudson Bay (SHB and WHB), thick-billed murre (*Uria lomvia*) from Coats Island, and caribou (*Rangifer tarandus*) from a WHB herd. The Hudson Bay region is a contamination hotspot in the Canadian Arctic and climate change effects may be more evident in polar bears and seabirds in these more southerly latitudes and ranges relative to other subpopulations. Concentrations of THg, *p,p*'-dichlorodiphenyltrichloroethane (DDE), the total of five PCBs (Sum_5PCB) as well as the individual congeners CB99, 138/158, 153, 170/190 and 180 were measured in seabird eggs and tissues of polar bears; mercury was also measured in caribou. The concentrations were corrected for biological covariates (diet, age) as necessary and were compiled from the early 1990's to present (2007-present for SHB polar bears). Climate factors included in statistical models included air temperatures, ocean temperatures, ice conditions, precipitation, and oscillation indices. Preliminary temporal analyses showed that THg concentrations decreased in SHB bears (-4.4%/year, insignificant, p >0.05) and caribou (-4.1%/year, $p < 0.05$), with no discernible trend in thick-billed murre eggs (-0.0022%/yr, p >0.05) and an increase in the WHB bears (+7.3%/year, $p < 0.05$). CB153 also increased significantly in WHB bears (+2.6%/year) while it decreased in seabirds (-4.2%/year, $p < 0.05$) and in SHB bears (-4.0%/year, p >0.05). In contrast, *p,p'*-DDE decreased in both seabird eggs and polar bears from WHB (-4.1 and -4.9%/year respectively), but increased slowly in SHB (+1.3%/year, p >0.05). Climate-related sources of the variation between these species and contaminants will be presented.

498 Temporal and geographic trends of perfluoroalkyl alkyl substances in Arctic Caribou and Reindeer

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Monitoring of arctic wildlife has shown that per- and polyfluoroalkyl substances (PFASs) are the most prominent persistent organic contaminants in terrestrial wildlife. The purpose of this study was to examine geographic and temporal trends by combining PFAS data for caribou and reindeer (Rangifer tarandus) from the Canadian Arctic, Greenland, Svalbard and northern Sweden. Results for PFASs in liver were available from 8 caribou herds (Canada, 6; Greenland, 2) and 5 reindeer herds (Greenland, 1;Svalbard, 1, Sweden 3) based on data from four laboratories. The predominant PFASs in Canadian caribou liver were the perfluorocarboxylates (PFCAs) with 9, 10 and 11-carbon chains (PFNA, PFDA and PFUnDA). Concentrations of PFNA and PFDA were above detection limits in almost all samples from all arctic herds. PFNA concentrations, (max 35 ng/g ww), were significantly higher in the Akia caribou from Western Greenland than all other herds except for the other Greenland herd (Kangerlussuaq). PFOS was the most dominant PFAS in caribou/reindeer from Greenland and Sweden. Highest concentrations were found in the two Greenland caribou herds and at the three Swedish sites. These five localities were statistically different from all other areas, but not each other. Highest concentrations were found in Kangerlussuaq (range 6.0-28 ng/g ww) and Akia (range 7.2-19 ng/g) herds. PFOS constituted above 50% of total PFASs in these herds. In Svalbard PFOS constituted ca 40% of PFASs and in the Canadian herds the proportion of PFOS was much lower, ranging from 0-20%. Concentrations of total PFCAs (C9 to C12 chains) and PFOS in the Porcupine and Qamanirjuaq herds appear to have declined by about 50% over the period 2005-08 to 2015. However, short chain (C4-C7 PFCAs) have increased over the same period. In conclusion, the results from the two Greenland cardibou herds were conspicuous compared to all other herds by having the highest concentrations of PFOS, PFHxS (only Kangerlussuaq), PFNA, PFDA, PFUnDA, PFDoDA (for the three latter only Akia had considerably higher concentrations). Both areas are relatively close to the international airport in Kangerlussuaq, while the range of the Akia caribou is adjacent to Greenland's capital city. Localized contamination could therefore play a role at these sites, However the main pathways of PFAS exposure for caribou/reindeer from remote areas most probably reflect atmospheric deposition and contamination of the Arctic terrestrial environment.

499 Climatic Influence on temporal trends of polychlorinated biphenyls and organochlorine pesticides in landlocked char from Canadian High Arctic Lakes

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Temporal trends and climate related parameters affecting the fate of legacy persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs; HCHs, DDTs and

HCB) and toxaphene were examined in landlocked Arctic char from six lakes in the Canadian Arctic located on Cornwallis Island (Resolute, Char, Amituk Lakes), one on Ellesmere Island (Lake Hazen), and two on Melville Island (West and East Lakes). Research performed over the past 10 years in the study area has revealed ongoing permafrost disturbances, which are of significant magnitude and importance in the West Lake watershed. Adult char were collected in late July from almost every year from 2001 to 2016, by gill netting or by jigging through the ice at a rate of 7 to 25 adult fish per lake and year. All fish were dissected in situ and subsamples of muscle+skin, liver, otoliths and GI tract were kept frozen for transport and storage in an ultra clean freezer (-30ºC). In total, more than 500 samples from muscle+skin arctic char were collected and analyzed for POPs in this study with the aim of i) examining the trends of legacy POPs, ii) studying which biological parameters and lake characteristics are influencing the bioaccumulation of POPs in Arctic char and iii) investigating whether or not climatic parameters and climatic oscillation patterns may affect the temporal trends of POPs in Arctic char. Overall, among biological parameters, lipid content was a key factor explaining the concentration of most POPs in Arctic char. A significant decline in the lipid content was observed in char collected at West Lake likely due to the stressors and limited access of char to food supply in this lake. Legacy PCBs and OCPs generally showed declining trends of concentrations in char as expected due to the past national and regional bans and restriction on uses and emissions of in circumpolar and neighboring countries. However, fluctuations in concentrations of POPs in char from the last two decades correlated with interannual variations of the North Atlantic Oscillation (NAO). Concentrations of POPs in char from the most remote lakes were enhanced during positive NAO phases. This relationship with NOA may reflect shifts in the hydrological cycles of the lakes and shifts in fish diets. Arctic warming, which is causing disturbances in permafrost and increased organic carbon inputs to lakes, appears to be influencing the health and trends of POPs in Arctic char.

500 Community based seawater monitoring for organic contaminants and mercury in the Canadian Arctic

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In Arctic marine ecosystems numerous emerging and legacy organic contaminants and methylmercury (MeHg) are bioaccumulated from seawater to lower food web organisms and may reach elevated levels in top predators, such as seals and polar bears, as well as humans consuming them as part of a traditional diet. To date, most contaminant measurements in Arctic seawater have been ship-based and are therefore limited to open water conditions and have not examined seasonal variability, including effects of ice cover and snow/ice melt. In 2014, we established a community based seawater monitoring program to examine contaminants with varying physical/chemical properties including perfluorinated alkyl substances (PFASs), brominated flame retardants (BFRs) current use pesticides (CUPs), organophosphorus triesters (OPES), and MeHg. Sampling is being carried out in May under ice and in August in open water conditions in Barrow Strait near Resolute, Nunavut and inAnaktalak Fiord near Nain, Labrador. Collections are being carried out using Niskin samplers at 5 depths of the ~100 m water column, large volume sampling through a XAD resin column/filter, and "passive" methods (polyethylene membranes deployed for ~5 weeks). Results to date show that near Resolute Bay, PFOS concentrations have declined steadily since 2008 and are now non-detect (< 1 pg/L). Concentrations of brominated flame retardants were low while phosphorus based flame retardants were detected for the first time at this location. Concentrations of 9 OPEs were lower (< 0.01-1.5 ng/L) compared to ship-based sampling in the Arctic (14-36 ng/L) and were dominated by TBEP, TPP and chlorine containing OPEs, TCPP and TCEP. Seawater MeHg concentrations were comparable to those reported for 2004-05 (range ~20-100 pg/L) and were higher in surface waters in May than in August, likely due to losses in the open water season via photo-degradation. Results for CUPs will also be presented, as well as comparison of polybrominated diphenyl ethers (PBDEs) using "passive" and "active" methods. Overall, results to date highlight the need to include examination of temporal trends that consider seasonal variability.

501 Twenty Seven Years of Mercury Concentrations in Arctic Char (Salvelinus alpinus) from the High Arctic: Temporal Trends and Relationship to Climate

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The Arctic is being subjected to a multitude of anthropogenic stressors, often at greater levels than the lower latitudes. Fossil fuel emissions have both warmed the climate and increased deposition of fuel-based contaminants, both of which have impacted Arctic people and ecosystems. Mercury deposition to high latitudes has led to accumulation of the neurotoxic form of mercury, methyl mercury (MeHg) in animals, compromising the quality of the subsistence diet of indigenous people. Several of the steps in the MeHg bioaccumulation pathway, which results in high concentrations of MeHg in top predators, are sensitive to climate. The objective of this study was to ascertain whether climate variables are related to trends in MeHg in landlocked arctic char (*Salvelinus alpinus*), the top predator, in five lakes near the community of Resolute Bay, Cornwallis Island, Nunavut; several of which are subsistence fisheries for the community. Char were collected annually from five lakes by gill netting or jigging through ice, immediately measured, dissected, and kept frozen prior to subsampling of skin on filets and total Hg analysis by DMA or CVAAS. The resulting data set spans 27 years and includes 842 individuals, with an average catch/year of 10.4 char for each lake. The trends of MeHg concentration for each population were modeled using segmented linear regression. The MeHg concentration data for all the populations was then pooled and regression modeling was conducted using concurrent climate and sea ice data. All five populations reached a peak in MeHg concentration in 2005, since this year, four have declined or leveled off and one is increasing. A strong statistical relationship between MeHg in char muscle and the climate variables sea ice duration (+), summer rain (-), spring temperature (+) was found. These climate variables likely influence mercury methylation rates in sediments, photodemethylation, dissolved mercury photoreduction, as well as char feeding habits and growth rates. The common breakpoint in the MeHg records across lakes followed by both increasing and decreasing trends demonstrates both the sensitivity of the arctic char as a sentinel for mercury monitoring and the capacity for divergent responses to mercury inputs across watersheds /populations, highlighting the need for continued mercury monitoring in the high Arctic.

Great Lakes Restoration Initiative – Occurrence and Effects of Contaminants of Emerging Concern – Part 2

502 Environmentally Derived Urban Mixtures of Contaminants of Emerging Concern Alter Reproduction in Fathead Minnows Over Three Generations

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The Laurentian Great Lakes contain 21% of the worlds fresh water, but most of its tributaries receive complex mixtures of contaminant of emerging concern from various sources that ultimately drain into these lakes. Little is known about how environmentally relevant concentrations of a CEC mixture affect fish over multiple generations. In this study, we exposed three generations of fathead minnows (F1 [adults], F2 [entire lifecycle], and F3 [early development]) to a mixture of Galaxolide (synthetic musk), TBEP (plasticizer), Estrone, Bisphenol-A (Plasticizer), DEET, Methyl-1H-benzotriazole (anti-icing agent), Desvenlafaxine (anti-depressant), Fexofenadine (allergy medication), Metformin (diabetes medication), and Nonylphenol at three concentrations that reflect environmental occurrence of these compounds. A multitude of endpoints were measured to assess the effects of the urban mixture on fish health and development. Using a repeated measures ANOVA and Holm-Sidak post-test, F1 and F2 adults had significantly higher fecundity rates at the Low and Medium treatments than High and EtOH control treatments ($p< 0.0001$, $F=34$ and 32 respectively, $df=3$). Low treatment F1 males were more dominant (higher secondary sex characteristic score) than EtOH, Medium, and High treatment F2 males ($p=0.01$, 0.006, 0.0007, Generalized Linear Model). F3 larvae had a slower total escape response compared to F2 larvae ($p< 0.001$, Generalized Linear Model). A generational effect was found in larval feeding, with F3 larvae eating 60% more than F2 larvae ($p=0.003$, Generalized Linear Model). This study provides evidence that exposure to the CEC mixture can alter fecundity and inhibit larval predator avoidance performance with, as of yet, unknown consequences for fish populations.

503 Exposure to a Complex Agricultural Mixture Alters Reproduction in Fathead Minnows Over Multiple Generations

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In aquatic ecosystems, organisms are exposed to complex chemical mixtures throughout life, producing effects not anticipated in laboratory settings designed to test acute effects of single chemicals. By exposing fathead minnows through three generations, we aim to capture exposure during sensitive life stages otherwise unobserved during acute exposures. We analyzed the effects of eight common co-occurring chemicals at environmentally relevant concentrations in the Great Lakes watershed in areas of predominantly agricultural land use. Minnows were housed in a flow-through exposure system and propagated for three generations (~10 months). Larval fish were analyzed for predator-avoidance behavior, feeding efficiency, and growth. Adult fish were analyzed for fecundity, biological indices, and hematological characteristics (VTG, glucose). Larval fish demonstrated no changes to predator-avoidance behavior and feeding efficiency, but significant differences in growth occurred, possibly as a result of either a therapeutic hazard effect or due to density-dependent growth. Adult fecundity was significantly decreased at increasing concentrations, potentially due to increasing chemical stress on the fish. No significant differences to morphological indices occurred. Significant increases in male plasma VTG concentrations were observed at the highest concentration, due to the estrogenic nature of some mixture chemicals. Results indicate that mixtures, environmentally relevant in composition and concentrations, have the potential to alter growth, lead to reductions in fecundity, and elevated egg-yolk precursor protein in male fish. Further research will determine whether organismal effects alter population levels, and whether behavioral responses reduce nest defense and reproductive behaviors of male fathead minnows.

504 High-resolution Mass Spectrometry of Skin Mucus for Monitoring Physiological Impacts in Fish Exposed to Wastewater Effluent at a Great Lakes AOC

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High-resolution mass spectrometry is advantageous for monitoring physiological impacts and contaminant biotransformation products in fish exposed to complex wastewater effluent. We evaluated this technique using skin mucus from male and female fathead minnows (*Pimephales promelas*) exposed to control water or treated wastewater effluent at 5%, 20%, and 100% levels for 21 days, using an onsite, flow-through system providing real-time exposure at the Western Lake Superior Sanitary District (WLSSD) wastewater treatment plant. Both sex-specific and non-sex-specific responses were observed in the mucus metabolome, the latter suggesting the induction of general compensatory pathways for xenobiotic exposures. Altogether, 85 statistically significant treatment-dependent metabolite changes were observed and 30 of those were annotated with probable structures. The *mummichog* software package was used to elucidate impacted biochemical pathways and to enhance metabolite annotation. Partial least squares regression models revealed relationships between the mucus metabolomes and upregulated hepatic mRNA transcripts reported previously for these same fish. These regression models suggest that mucus metabolomic changes reflected, in part, processes by which the fish biotransformed xenobiotics in the WLSSD effluent. Further, we detected a phase II transformation product of bisphenol A in the skin mucus of male fish. Collectively, these findings demonstrate the utility of mucus as a minimally invasive matrix for simultaneously assessing exposures and effects of real-world mixtures of contaminants.

505 Place-based screening of mixtures of dominant emerging contaminants measured in Lake Michigan using zebrafish embryo gene expression assay

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Determining the impacts of chemicals such as emerging contaminants are difficult due to the different concentrations of mixtures of these chemicals over a landscape. Both in vitro and in vivo effect-based assays have shown promise for water quality assessment. To be useful assessment purposes these approaches need to account for ADME of the chemicals in an organism, and crosstalk between molecular pathways, which may lead to an unforeseen induction of an adverse outcome pathway. The goal of this study was to assess the utility of employing the zebrafish (ZF) in a modified FET that assesses morphological alterations and measurements of estrogen-associated mRNA transcripts to exposure mixtures of chemicals at concentrations measured in several locations in Lake Michigan, USA. ZF embryos were exposed from 6 hpf to 144 hpf to one of three exposure types: five individual chemicals identified from a previous study in our lab (Blair et al. 2015), mixtures, and a positive control. The 5 pharmaceuticals in this study were carbamazepine, diltiazem, fluoxetine, gemfibrozil and metformin. Exposures consisted of 4 concentrations of each individual chemical, 7 different mixture concentrations measured at seven separate locations in Lake Michigan, or 4 concentrations of 17ß-estradiol. Mortality, hatching rates and observed developmental malformations in ZF were compared across treatments. The relative expression of estrogen receptor alpha, brain aromatase, and GnRH3 mRNA were measured at the end the 5-d exposure. Dose-response for each mRNA transcript was compared against the 4-point dose-repose curve generated from an exposure to 17ß–estradiol. There was significant induction of brain aromatase in individual exposures of diltiazem, fluoxetine, gemfibrozil and metformin at concentrations measured in Lake Michigan. Exposure to 5 of the 7 chemical mixtures representing concentrations measured in Milwaukee harbor and Lake Michigan altered the expression one of the three biomarkers with transcript response varying across sites. This research demonstrates that measures of multiple molecular endpoints measure in the ZFET assay in conjunction with chemical measurements could provide greater information as to the toxic potential of these chemicals in environmental monitoring efforts.

506 Proteomics analysis of the lake trout (Salvelinus namaycush): A comprehensive approach

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Lake trout have been utilized by the Great Lakes Fish Monitoring and Surveillance Program (GLFMSP) to monitor toxics for decades. Volumes of data have been published on the concentration of polychlorinated biphenyls and organochlorine pesticides. The exposure of these xenobiotics affects the genes that are transcribed, mRNAs that are translated and proteins that are produced and which are later post-translationally modified. There is currently very little biochemical information on the proteome of this species. In the current study, we use a biochemical, proteomics and bioinformatics approach to identify the proteomes from liver, heart, brain, blood and body of the lake trout. Tissues from different organs were homogenized and then separated by SDS-PAGE, followed by the in-gel trypsin digestion and nanoLC-MS/MS analysis on a NanoAcquity UPLC coupled to a Xevo G2 Q-TOF mass spectrometer (both from Waters). Raw data was converted to pkl files using PLGS 2.4 software (Waters), which were then submitted to database search using Mascot server (Matrixscience). The database search was performed against all fish species (including the most comprehensive Danio rerio) and accompanied by a de-novo database search. The output from the Mascot server was then submitted to Scaffold 4.3 software (Proteome Software) for an organ specific classification of the proteins and for differential analysis among the various organs. The methodology and progress of building a comprehensive proteomics database will be discussed with the end goal to explore the biochemical perturbations in lake trout related to toxic chemical burden will be discussed.

507 Predicting CEC Occurrence for Great Lakes Tributary Watersheds Using Boosted Regression Tree Methods

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The U.S. Fish and Wildlife Service initiated a collaborative study with the U.S. Geological Survey to characterize and evaluate the risks contaminants of emerging concern (CECs) pose to fish and wildlife resources in the Great Lakes Basin. From 2010 through 2014, water samples were collected from 24 Great Lakes tributaries at more than 200 sites. Samples were analyzed for a broad suite of CECs including hormones, pharmaceuticals, alkylphenols, pesticides, fragrances, and fire retardants. Preliminary statistical analysis of the chemical results using multi-variate methods showed complex mixtures of pharmaceuticals, hormones, and fire retardants were associated with point sources (e.g. wastewater treatment facilities and combined sewer outfalls) and urban land use. Boosted regression tree (BRT) analyses were used to develop predictive models to better understand relationships between chemical class occurrence and watershed characteristics. Chemicals detected in >30% of all samples were placed into one of eleven chemical classes and analyzed as a binomial (absence / presence) variable by site. Twenty-six upstream watershed characteristics were derived for three spatial scales (90m buffer, 250m buffer, and entire basin) from NLCD (2011), TIGER (2015), and NHDPlus data as well from USEPA and ESRI sources. Results from a single spatial scale were used as independent variables in BRT model development. Based on total accuracy of predicting absence as well as presence, acceptable BRT models were developed for a number of chemical classes including polycyclic aromatic hydrocarbons, organo-halides, pharmaceuticals, alkylphenols, and fragrances. Total accuracy of acceptable BRT models ranged from 68 to 85%. Development of accurate models was achieved through some degree of over-training of the data sets, but BRT model results were in agreement with significant logistic regression (LR) models ($R2 > 0.45$; $p < 0.001$) for the same dataset. All 26 watershed characteristics were significant predictor variables in at least one BRT and one LR model. Among the 26 characteristics, percent of the watershed containing developed landuse and distance to point sources were often important variables in predicting the presence of chemical classes. Although limitations exist, these predictive models can be one tool to inform vulnerability assessments of specific sites to CEC occurrence and to help direct fish and wildlife management efforts.

508 Evolution of Lampricide Resistance to TFM Is Not Related to Temporal History of Exposure

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Invasive sea lamprey (*Petromyzon marinus*) – hematophagous parasites that prey upon a variety of fishes – represent a major threat to the Great Lakes ecosystem. For the last 60 years, the primary method for controlling sea lamprey has been to target larval sea lamprey through the application of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) in streambeds of Great Lakes tributaries. TFM application has been effective at reducing sea lamprey population sizes in the Great Lakes (i.e., field estimates of ~90% mortality in streambeds after application). High but incomplete mortality associated with exposure suggests that TFM may be a strong selective agent, which could result in rapid evolution of genetic resistance. The goal of this study was to determine if sea lamprey populations with a history of exposure to TFM are evolving resistance to lethal and sublethal concentrations of the lampricide. One possibility is that the evolution of resistance is directly related to the length of time a population has been treated with TFM. To test this hypothesis, larval sea lamprey were collected from locations where TFM treatments have been conducted for 0 (native range in Connecticut), 30 (Lake Champlain), and 60 (Lake Michigan) years. Collected lamprey were subjected to lethal and sublethal toxicological assays over a 12-hour period simulating a typical TFM stream exposure. Lethal effects of TFM were measured via hourly mortality surveys. Because TFM is thought to uncouple mitochondrial oxidative phosphorylation and increase oxygen consumption, sublethal effects were quantified by measuring respiration rates of treated and control individuals from each location at the conclusion of the exposure. Our results provided no evidence of resistance to either lethal or sublethal concentrations of TFM. We observed no relationship between the number of years a location was treated and ammocoete survival, and individuals from the native 'naïve' location surprisingly demonstrated the highest survival. Additionally, while differences in respiration rates were observed between exposed and unexposed individuals from a given location, there was no evidence of a location by treatment interaction indicative of resistance evolving in one (or more) locations. While our results suggest that resistance to TFM has not yet evolved, additional studies are required to corroborate these findings before decisions can be made regarding future sea lamprey control and management.

509 From simple to complex: Behavioral changes in fish exposed to contaminants of emerging concern singly and in mixture

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Contaminants of emerging concern (CEC), including pharmaceuticals, personal care products and industrial agents may impact aquatic life. Previous studies have documented reduced escape performance in fathead minnow larvae exposed to diverse CECs. However, these studies did not consider the complex mixtures of CEC present in the environment. The current study tested the hypothesis that CECs in mixture change fathead minnow behavior differently than the mixtures' individual constituent. We assessed the potential of 20 commonly detected CECs to alter both juvenile (escape performance, feeding efficiency) and adult (nest defense, courtship, boldness) behaviors central to survival and reproduction after 96-hour flow-through exposures. In addition, we began the process of building increasingly complex mixtures of CEC using the same compounds. Compound concentrations and mixture composition were based on an analysis of nearly 500 water samples collected as part of the Great Lakes Restoration Initiative. Results to date suggest changes in survival, escape performance and feeding efficiency. The survival was significantly reduced ($p< 0.05$, ANOVA) in larvae exposed to atrazine, metolachlor, and N, N-Diethyl-m-toluamide (DEET). In both metolachlor and DEET exposures, the medium concentration (environmental concentration) had significantly more non-responsive larvae as part of the escape performance assay, as was the case in the highest concentration (10x) of atrazine exposure. Interestingly, several treatments increased the feeding efficiency of larvae under chemical stress. These results show that herbicides have strong effects on larval survival. We expect that these evaluations will lead us to understand the effect of CECs in mixture on fish survival and behavior.

Systems Biology for Ecotoxicology – From Gene to Ecosystem – Part 2

510 Developing an integral projection model to inform grass carp management

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Population models are used as part of ecotoxicology to guide control efforts and understand the impacts of toxicants and other stressor. These models provide insight into the population-level impacts of stressors and control actions and can guide management (e.g., how much control effort is necessary for a method to work?). One important modeling assumption is how a modeler deals with the size of individuals. Traditionally, matrix models have discretized size into size classes, but this can produce modeling artifacts and dynamics that are driven by the number of size classes. Integral projection models avoid this modeling artifact and treat size as a continuous variable. We developed an integral projection model for grass carp, an invasive species that causes ecological destruction and mayhem in North America. We specifically explore the use of YY-males as a control method for grass carp populations. YY-males are male fish that only produce male off spring. We found that YY-males would likely not be reasonable based upon the number of YY-males required to grass carp populations.

511 Integrated transcriptional-regulatory and flux analysis models of piscine steroidogenesis

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Within living systems the difficult task of multi-level integration, from genome to phenotype, is accomplished using interrelated biological networks, such as: transcriptional-regulatory (gene-protein) and metabolic networks (protein-metabolite). The coordinated interactions of such networks yield physiologically relevant functional states, with perturbed fitness arising when various combinations of networks are disrupted by environmental and/or anthropogenic stressors. Therefore, quantitatively associating underlying transcriptional-regulatory control with metabolic network function is essential for unifying multi-leveled biological complexity and brings us closer to a predictive toxicology paradigm. This presentation will showcase the use of stoichiometric matrix models to study the functional properties of transcriptional-regulatory and metabolic networks. Specific examples of modeling piscine reproductive endocrine developmental programming and steroidogenesis will be presented. *First*, 'synchronization' between brain and gonad reproductive endocrine transcriptional-regulatory networks was discovered in developing zebrafish from 10-12 hours post fertilization (embryonic life-stage) to 30-90 days post fertilization (adult life-stage). *Second*, recent progress with integrating transcriptional-regulatory control with piscine reproductive steroidogenic flux distributions will also be presented. The work presented is a step towards the construction of integrated models that bridge the transcriptional/metabolic landscapes with regulatory-relevant apical or physiological endpoints.

512 Transcriptomic response to early life-stage exposure to atrazine or 17α-ethinylestradiol in male and female largemouth bass (Micropterus salmoides)

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Field studies have shown disease outbreaks and a high prevalence of intersex in smallmouth bass (*Micropterus dolomieu*), and to a lesser extent largemouth bass (*Micropterus salmoides*), populations in the Chesapeake Bay watershed. The intersex phenotype has been correlated with the presence of the agricultural pesticide atrazine, which is widely used and one of the most commonly detected pesticides in the watershed. Our objective was to investigate alterations in key molecular events during gonad formation in largemouth bass (LMB) exposed to either atrazine (1 or 100 µg/L) or the model estrogen 17α-ethinylestradiol (EE2; 1 ng/L). Fry were exposed directly after hatch, from 5 days post spawn (dps) through early gonad development (80 dps). Transcriptomic effects in the gonad of these fish were assessed via RNAseq, using the Illumina NextSeq platform. The samples clustered together in four distinct groups: (1) female solvent control, 1 µg/L atrazine, and 100 µg/L atrazine, (2) female 1 ng/L EE2, (3) male solvent control and 1 µg/L atrazine, and (4) male 100 ug/L atrazine and 1 ng/L EE2. There was a particularly robust response in males treated with 100 ug/L atrazine and 1 ng/L EE2, with 2037 and 3811 differentially expressed (DE) contigs, respectively. Of these, 1464 contigs were common among both treatments. Male LMB exhibited a greater number of DE genes in response to all three treatments, compared to female LMB. There was little overlap among DE genes in males and females in response to the two atrazine treatments, indicating sex-specific responses. However, in the EE2 exposed fish, of those DE contigs (3811 in males, 2120 in females) 721 were identified in both sexes. There was also a strong correlation of log fold changes between males and females in these 721 contigs ($R^2 = 0.92$), suggesting that some pathways responsive to EE2 exposure are not sex-specific. Transcriptomic analysis is challenging for a non-model species that does not have a fully annotated genome. However, even based on analysis limited to zebrafish homologs, there was overrepresentation of genes from immune response, steroidogenesis, and lipid metabolism pathways. Further analysis is being conducted to delineate the specific drivers of the common response in EE2 treated males and females as well as the atrazine and EE2 treated males, which will shed light on the mechanisms by which these contaminants can effect gonad development.

513 Impacts of crude oil on marine pelagic fish: From molecular and cellular responses to habitat utilization of wild mahi-mahi (Coryphaena hippurus)

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Mahi-mahi are high performing pelagic fish. This is exemplified by measurements of their maximal cardiac performance, maximal oxygen uptake rates, and maximal systained swim speeds which are among the highest recorded of any fish. In additon, mahi-mahi have wide geographical distribution, and are ecologically and economically important. Early life stages of mahi-mahi are, as expected, highly sensitive to polycyclic aromatic hydrocarbons (PAHs) present in crude oil with metabolic rates, metabolic buoyancy control, and development being impacted during sublethal exposures (low ppb range). In contrast to expectations, later life stages are surprisingly sensitive to even brief exosures to PAH concentrations in the low ppb range. RNAseq data has revealed, among other things, that peripheral nervous systems and cellular calcium cycling pathways are predominant targets of oil exposure. Phenotypically, later life stages exhibit impaired sensory functions and reduced swim performance. Reduced swim performance is associated with reduced maximal oxygen uptake and reduced aerobic scope that is dictated by reduced cardiac output in oil-exposed fish. The reduced cardiac output is mainly attributable to reduced stroke volume, which in turn can be explained by reduced sacromere shortening in freshly isolated cardiomyocytes exposed to oil. Such reductions in cardiomyocyte contractility are likely related to impaired cellular calcium cycling also suggested by RNAseq data. Pop-up satellite tag data from wild, adult mahi-mahi has revealed that these highly aerobic fish perform extensive migrations of up to 100 km/day. Experiments to address how sublethal impacts of oil exposure on sensory systems and swim performance may influence migration, foraging and spawning behavior by mahi-mahi in the wild are currently underway. Possible alterations to the behavior of wild mahi-mahi following oil exposure could impact ecosystems throughout the vast geographical range of this ecologically important species. This research was made possible by a grant from the Gulf of Mexico Research Initiative. Grant No: SA-1520; Name: Relatioships of Effects of Cardiac Outcomes in fish for Validation of Ecological Risk (RECOVER).

514 Ontology-based Semantics Mapping and Its Applications in Toxicological Data Mining

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An ontology could be considered as a data graph, where nodes and their inter-connecting edges come from terms in a logically controlled and predefined vocabulary. The knowledge in a given domain, for example genes, anatomies, and phenotypes, can be captured by these terms. Multiple ontologies spanning diverse knowledge domains can be integrated to enable semantic analysis among selected parts of this ontology graph. Of particular interest to toxicological data mining are various phenotype ontologies built from data generated by a number of ongoing public phenomics (high throughput phenotyping) efforts in several vertebrate species. Taking advantage of these publicly available ontologies and several open-source software tool kits, we have implemented an OS-Mapping (Ontology-based Semantics Mapping) approach as a Java application, and tested its utilities in constructing putative adverse outcome pathways, extending chemical-impacted molecular targets to their higher level phenotypes, and evaluating chemical toxicity across various vertebrates including fish, mouse, rat, and human. The test data originated from two sources. The first is EPA ToxCast high throughput chemical screening project (https://www.epa.gov/chemical-research/toxicity-forecasting), where the October, 2015 release contains screening data of 375 unique target genes or proteins by 360 unique assays for over 9000 chemicals. Over 6500 chemicals were determined to be active on at least one target. The second source is the EPA EcoTox database (https://cfpub.epa.gov/ecotox/). It contains curations of published literature from exposure studies covering over 7000 chemicals among 1600 taxa. Taking phenotypic profiles each made up of multiple ontology terms as input, we demonstrated that our OS-Mapping tool is able to link chemical-impacted molecular targets to a variety of phenotypes, to construct a network of phenotypes anchored on genes, diseases, and chemicals, and to evaluate chemical toxicity semantically across several vertebrate species of interest.

515 Forecasting Population-Level Impacts of Diverse Chemicals with a Quantitative Adverse Outcome Pathway Model for Aromatase Inhibition in Small Fish

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A great challenge for systems toxicology is to extend the more conceptual adverse outcome pathway (AOP) framework into a quantitative model capable of correlating a degree of molecular initiation with population level effects stemming from individual adverse outcomes. Despite that a great wealth of mechanistic data is usually required to develop and validate models for specific chemicals, no methods have yet been developed that can rigorously transform such chemical-specific models into the chemical-independent setting of the AOP framework. Here we report a breakthrough of this problem obtained by using a nonlinear toxic equivalency factor (nTEF) approach to correlate the activation of molecular initiating events (MIEs) by comparing different chemical data from ToxCast assays. This approach was applied to a mechanistic mathematical model of fathead minnow fecundity we developed and trained from a genomics analysis of tissues collected from female fathead minnows (Pimephales promelas) exposed the nonsteroidal aromatase inhibitor fadrozole. The model incorporates androgen control of phosphorylation of the cAMP response element binding (Creb) and Paxillin proteins in granulosa cells of ovary tissue, which promote de novo aromatase synthesis to compensate for inhibition by fadrozole, in addition to transcriptional regulation of the steroidogenic acute regulatory (Star) protein, which, in turn, controls the abundance of androgen substrate through steroidogenesis. At the highest concentration of fadrozole tested experimentally, spawning was curtailed so the case study was able to explore how mild to severe reductions in fecundity affected population dynamics. Consistent with the expectations for a qAOP, our model can potentially be used to evaluate these effects for endocrine disrupting compounds (EDCs) beyond fadrozole. We evaluated this capability by applying the nTEF approach to steroidogenic enzymes in our qAOP model of the fathead minnow for exposure data from the fungicide imazalil.

516 The development of a biological response model using transcriptomic changes in Isidorella newcombi exposed to copper

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Advances in molecular technologies have increased our ability to understand responses of organisms to contaminant exposure at a mechanistic level. It has been argued that the use of RNA-seq in organisms exposed to contamination allows; (i) the responses to contaminants to be detected early, (ii) changes in biological pathways associated with specific responses to be detected and (iii) an indication of the level of stress an organism is experiencing to be determined. RNA-seq also has the ability to detect novel functional pathways. Despite substantial potential, there are many challenges in establishing RNA-seq a reliable means of assessing responses to contamination. These include the need to use non-model species, link transcriptional changes to biological functions and establish contaminant-specific transcriptomic footprints. In this study, the response of the Australian endemic freshwater snail *Isidorella newcombi* to copper is used to develop a model for the interpretation of RNA-seq data from an ecotoxicology perspective. *Isidorella newcombi* is considered a pest in rice growing areas andis controlled with copper sulphate. *I. newcombi* were exposed to sublethal concentrations of copper and RNA-seq was performed. Following alignment and annotation of transcripts data, quantitative analysis was conducted between copper exposed treatments and controls. The differential responses between the control and copper exposed organisms were categorised as being associated with copper uptake and storage, survival mechanisms and cell death. Categorising transcriptomic responses into these categories provides the greatest opportunity to detect contaminant specific responses and detect changes that are indicative of organism condition. The broader adoption of such a framework in the interpretation of RNA-seq results for ecotoxicology studies would allow greater consistency and comparability betweeen RNA-seq studies in the area of ecotoxicology.

517 Evaluating in vitro–in vivo extrapolation success for a series of model endocrine active toxicants

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We have tested a suite of model toxicants using cellular in vitro assays based on the female rainbow trout pituitary, ovary and liver. Each assay measures an essential reproductive endocrine function such as estrogen synthesis and secretion by ovarian follicles. In vitro results are extrapolated to metrics of reproductive performance (fecundity, egg size) in trout using a mathematical model of the trout hypothalamus-pituitary-ovary-liver (HPOL) axis. These studies are part of a larger effort seeking the best approaches for quantitative in vitro–in vivo extrapolation (IVIVE) to support implementation of adverse outcome pathway (AOP) based toxicity testing. In the present study, we evaluated IVIVE by comparing predicted toxicant effects against laboratory results obtained from a yearlong exposure of female trout to four different chemicals (tamoxifen, prochloraz, fluoxetine and trenbolone). A single water exposure level was tested for each chemical, guided by preliminary studies and a desire to use a maximum tolerated exposure that still allowed spawning to occur. Laboratory exposures began 10 d after the first spawning cycle and lasted until time of ovulation and completion of the second spawning cycle. Trout were then euthanized and total fecundity determined along with egg mass and diameter, fertility, hatching success and larval growth. No effect on fecundity was observed except after the 75 ng/L trenbolone exposure, which caused regression of ovarian growth and failure to spawn. The most significant effect on egg quality occurred after the 501 ng/L tamoxifen exposure, which caused a 30% decrease in egg mass and diameter. The decrease in egg size translated to significantly smaller larvae at 20 dph (yolk-sac resorption). The HPOL model accurately predicted the effects of tamoxifen exposure on egg size, but only after consideration of formation of the 4-OH-tamoxifen metabolite, which is a more potent anti-estrogen. Supported by EPA-STAR grant R835167.

High-Technology Metals as Emerging Contaminants of Potential Concern – Environmental Fate, Transport and Toxicity

518 Interactions of lanthanum and cerium with a green alga: Uptake and toxicity

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Until very recently, little attention has been given to the ecotoxicology of rare earth elements (REEs). Despite a recent spurt of publications, there are still few data available for risk assessment purposes (exposure and effects). Of particular concern is the poor solubility of lanthanides in the presence of phosphate. Since algal growth requires phosphorus, toxicity assessment of REEs with photoautotrophs maybe be biased by the precipitation of phosphate-based mineral phases. The toxicity of La and Ce was evaluated at pH 5.5 using the unicellular green alga *Chlorella fusca* for which two exposure strategies were investigated. *Chlorella fusca* is an alga known to accumulate enough phosphorus to sustain its growth for several days in phosphate-free media. The first strategy was thus to determine growth inhibition of *C. fusca* caused by La and Ce in the absence of phosphorus for 120 h. The second strategy involved testing the cells in the presence of an organic source of phosphorus (β-glycerol phosphate) after determining that there was no significant metal binding by this organic source of phosphorus. Growth curves of *C. fusca* showed a decrease in the final yield of the cultures after five days which was used as an endpoint for toxicity assessment. In order to test for the influence of speciation, several ligands were used (iminodiacetate, nitrilotriacetate and malate). The observed EC_{50}s based on the calculated free ion concentrations were 440 ± 60 nM La^{3+} and 170 ± 60 nM Ce^{3+} and results indicate that accumulation and toxicity can be predicted based on the free La^{3+} and Ce^{3+} ion concentrations (metal complexation decreased uptake and toxicity). Using an organic source of phosphorus for the growth inhibition tests provided similar EC_{50} values. We also tested the influence of Ca as competitive ion which resulted in a decrease in uptake that allowed us to extract constants for the binding of Ca on the transporters used by La (log K = 4.26) and Ce (log K = 4.60). Calcium also provided a protective effect against La and Ce toxicity as expected based on the Biotic Ligand Model.

519 Transcriptomic signatures in Chlamydomonas reinhardtii as ionic Ce or CeO2 nanoparticle exposure biomarkers

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With the growing number of applications using rare earth elements (REEs), manufactured forms of these elements are expected to be released into the natural environment at increasing concentrations. Cerium (e.g. Ce) is one of the most used REEs. It is incorporated into several high-technology materials, including electronic applications (e.g. luminophore of LCD and plasma flat screens) and cerium dioxide in the form of micro- or nano-particles (e.g. CeO_2 NPs), used in polishing powders or for automotive catalysis (i.e. 44% and 13% of anthropogenic Ce, respectively). The bioavailability of the different forms of Ce for aquatic biota remains poorly elucidated, mainly due to analytical limitations resulting from the low exposure concentrations and high matrix complexity. Classical biological effects measurements of toxicity have generally shown few responses at environmentally relevant exposures of CeO_2 NPs, with little evidence for NP internalization by microorganisms. In this project, several Ce forms (ionic Ce; small CeO_2 NPs with different coating properties, i.e. uncoated, citrate or poly-acrylic acid coated) were investigated for their bioavailability to a unicellular freshwater alga, *Chlamydomonas reinhardtii*. Sub-lethal biological responses were analyzed by flow cytometry and whole transcriptome profiling analysis (e.g.

RNA-Seq) was determined for $[Ce]_{total} < 0.5$ μM at pH 7.0. Results have been interpreted with respect to bioaccumulation and speciation experiments performed in the same medium. Only the transcriptomic studies showed enough sensitivity to obtain significant biological signals at these sub-lethal exposure levels. Induction (or repression) was observed for a few 10's of genes for the citrate coated CeO_2 NPs as opposed to the ionic Ce or the uncoated and poly-acrylic acid coated CeO_2 NPs where expression levels changed significantly for hundreds of genes. The specific gene expression signals for each experimental condition suggested distinct mechanistic differences for the CeO_2 NPs as opposed to the ionic Ce. In spite of a low solubility of the CeO_2 NPs, higher or similar levels of Ce were internalized by the cells as compared to exposure media containing only ionic Ce. The combination of speciation, bioaccumulation and profiling analysis strongly suggested that all tested Ce forms were bioavailable to the algae.

520 A deeper ecotoxicological insight into REE exploitations' impact on aquatic biota

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Rare earth elements (REEs) have become a highly valuable commodity due to their increased use in high-tech products and medical applications. In the current geopolitical and economical context, (re)opening of mining facilities for REE extraction is being explored in several countries. Mining of REE may result in their dispersal from indigenous rocks to soils, sediments, water and possibly biota. In recent years, their environmental behavior has attracted increasing attention, studying their cycling and geochemistry, but their potential ecotoxicological effects require further investigation. Although they show the same behavior during natural processes owing to their similar chemical properties, there is no a clear consensus which support that they have also a coherent and predictable pattern of (eco)toxicity. This project focuses on if and how (re) opening of REE mines will alter the normal ecology of aquatic organisms at the sediment-water interface. We expect to establish a widely applicable conceptual approach to assess the environmental impact of REE mining and to estimate the maximum REE concentrations without impact on representative aquatic organisms. This research includes: a) characterization and sampling of a prospective REE-rich mining areas in Quebec with a 250 Mt carbonatite-hosted REE-Nb deposit; b) identification of the major properties that can affect REE availability in natural sediments; c) assess the release of REEs from enriched rock materials (especially ferro- and calcio-carbonatite, monazite- and allanite- bearing pegmatite); and d) study of REE toxicity to different test organisms. First results show that, although rocks in the studied area have REE contents up to 12,000 ppm, the content in adjacent sediment-water bodies does not necessarily match, which could imply a potential transport of REE. REE availability in sediments is very low compared to the total content and show correlations with grain size, content in Mg, Fe and Al, whereas eluviations derived from a rock-leaching process show contrasting toxicity results for the different aquatic organisms tested (e.g., *D. magna* had 100% of immobilization for the monazite rock using a liquid to rock ratio of 10 l/kg). Our findings suggest that REE could be leaching towards the sediment-water interface in mining areas. As they bear toxic effects more studies should be carried out to assess LN toxicity, and to define ecological safety levels.

521 Rare earth effects on microorganisms involved in anaerobic waste treatment

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Rapid growth of new energy technologies and consumer electronics is inducing increased fluxes of rare earth elements (REE), during the phases of resource extraction, product usage, recycling, and disposal. However, little is known about the impacts of these increased REE fluxes on environmental ecosystems. We have been evaluating the effects of rare earths on the function of an engineered ecosystem, namely anaerobic biological treatment. We found that europium and yttrium do not appear to affect methane production by anaerobic sludge consortia at nominal concentrations up to 600 μM REE. However, this gross measure may conceal impacts on individual members of the community. With pure laboratory cultures of *Sporacetigenium mesophilum*, a fermenting bacterium originally isolated from an anaerobic digester, we observed that Eu at nominal added concentrations of 6 and 60 μM in fact stimulated hydrogen production and also increased cell growth. By 600 μM Eu the stimulatory effect seemed to subside and by 2 mM Eu the metal additions appeared toxic. Effects of yttrium on *S. mesophilum* cultures were less clear. In some experiments 60 μM Y appeared to enhance hydrogen production, but in other experiments hydrogen production was inhibited. Analogous to the situation with Eu, nominal added Y concentrations of 2 mM inhibited hydrogen production completely. The mechanisms by which these metals exert their effects are unknown but present an intriguing target for further investigations. This work contributes to the assessment of the potential impacts of increased REE recycling and processing on ecosystems, and supports decision making with respect to disposal of wastewaters generated during these industrial practices.

522 Biogeochemistry of seleniferous mine soils: Metal(loid) impacts on microbial community ecology

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Worldwide, selenium (Se) is proving to be a significant environmental concern, with many anthropogenic activities (e.g., coal mining and combustion, phosphate mining and agricultural irrigation) releasing potentially hazardous concentrations into soil and natural water ecosystems. Specifically in southeastern Idaho, historic and on-going phosphate mining have resulted in Se-enriched soils throughout the region. Such metal(loid) contaminated environments have frequently been shown to have altered soil microbial community structures, though few studies have investigated the interaction of Se with aerobic soil microbial communities. Numerous anaerobic bacteria are known to mediate Se redox reactions in anoxic environments, and recent work has shown that fungi can aerobically reduce Se oxyanions to elemental Se and volatile Se(-II). The goal of this study is to investigate the aerobic microbial (bacteria and fungi) communities present in Se-containing surface soils, and the role these communities have on Se geochemistry in these soils. Both culturable and non-culturable microbial communities were assessed in soils collected from two reclaimed mine soils in southeast Idaho, USA. Additional unmined reference soils near the mines were also collected and analyzed. Soils were collected over the course of two years, and total microbial DNA extracted. Amplicon sequencing of bacterial and fungal DNA was performed using 16s rRNA gene sequencing (bacteria) and internal transcribed spacer (ITS) sequencing (fungi). Results show that the bacterial community is relatively similar across all sites (mined and unmined) though fungi in reclaimed mine sites have substantially greater relative contribution of Ascomycete fungi than Basidiomycete fungi. Additionally, increasing Se concentration in the soils was linked to lower overall fungal community diversity, while other geochemical parameters such as pH were not correlated at all to microbial community diversity. Understanding the microbial ecology in seleniferous soils will help to address Se-contamination and limit Se mobility and toxicity in sensitive environments.

523 Evaluation of the Direct Peptide Reactivity Assay (DPRA) to Assess Sensitization Potential of Platinum Species

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Platinum salts are among the most potent sensitizers, leading to occupational asthma in a large proportion of workers in the platinum refining and catalyst production industry. The Direct Peptide Reactivity Assay (DPRA) was developed in response to the need for a high throughput, animal-free method to screen for compounds with sensitization properties. The key molecular initiation event in the adverse outcome pathway for sensitization is the covalent binding of an electrophilic substance to a nucleophilic peptide (e.g. cysteine and lysine) within a protein. Therefore, DPRA measures the depletion of a synthetic heptapeptide, containing either cysteine or lysine, due to covalent binding of a test chemical to the peptide. Several concentrations of the test chemical are incubated with the synthetic peptide and the percent of peptide depletion is measured using liquid chromatography tandem mass spectrometry (LC-MS/MS). If depletion occurs, the concentration of the test chemical causing 15% depletion of peptide (EC_{15}) is reported. Of the platinum salts tested with the cysteine peptide, potassium hexachlorplatinate had the lowest EC_{15} of ~ 1 µM, followed by potassium tetranitroplatinate (EC_{15} ~ 1.5 µM), potassium tetrachloroplatinate (EC_{15} ~10 µM), cisplatin (EC_{15} ~ 30 µM), tetraamineplatinum dichloride (EC_{15} ~600 µM), hydrogen hexahydroxy platinate (EC_{15} ~20000 µM), and tetraamineplatinum nitrate (EC_{15} > 30000 µM). Results with the lysine peptide were less reproducible and in general indicated higher EC_{15} than results with the cysteine peptide. Studies indicate that occupational tetraamine platinum dichloride exposure does not result in sensitivities. Thus, results indicating the relatively low EC_{15} of this compound led to further exploration. Contamination of the standard was explored using new source standards and clean up with ion exchange columns. The solubility of test compounds is a limitation of the DPRA assay; therefore reactivity of test compounds under different pH and buffer components was also explored. Our work also evaluated the potential use of the DPRA to determine the reactivity of unknown compounds. IC-HPLC runs of chloroplatinate species incubated in KCl or EDTA resulted in additional platinum containing peaks. The fractions containing the peaks were collected and evaluated with the DPRA. Some fractions containing unidentified platinum species exhibited similarly high levels of reactivity as the parent compounds.

524 Widespread atmospheric Tellurium contamination in industrial and remote regions of Canada

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High tech applications, primarily photovoltaics, have greatly increased demand for the rare but toxic element Tellurium (Te). Here we examine 22 dated lake sediment Te concentrations profiles collected near: base metal smelters, coal mining/combustion facilities, oil sands operations, rural regions and remote natural areas of Canada. Te contamination was most prevalent near a Cu/Zn smelter but was also present in locations remote from anthropogenic activities in central and eastern Canada. From the sediment core records near the Flin Flon Manitoba Cu-Zn smelter, we estimate 72 metric tons where deposited within a 50km radius between 1930-2009. From this we deduce an emission factor of 21g of Te atmospherically deposited near Flin Flon per metric ton (t) of Cu processed. Furthermore, as net 1900-2010 global Cu production (minus production from recycling) is $451x10^6$ t, we estimate that 9,600 t of Te has been deposited near Cu smelters globally. Comparable to net global refined Te production since 1940. In a remote area of central Canada (the Experimental Lakes Area; ELA), pre-industrial Te deposition rates were equivalent to the estimated average global mass flux supplied from natural sources, while modern Te deposition rates were 600% higher and comparable with Te measured elsewhere in precipitation. We therefore suggest that sediment cores reliably record atmospheric Te deposition and that anthropogenic activities have significantly augmented atmospheric Te levels. Lake water residence time was found to influence lake sediment Te inventories among lakes within a region. The apparent settling rate for Te was calculated and was comparable to macronutrients (C, N, P), likely indicative of significant biological processing of Te.

525 The geochemistry and toxicology of rare earth elements

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The growing use of rare earth elements (REEs) in personal electronic devices, green technologies and medical applications results in a developing concern for impacts in aquatic environments. However, there are no water quality guidelines/criteria for REEs and few studies available. The overall objective of this research is to contribute data towards the establishment of assessment tools for the effects of REEs. We have studied the toxicity of Ce, Sm, Dy and Tm to fish and sensitive invertebrates (e.g. *Hyalella azteca* and *Daphnia pulex*). The toxicity modifying influences of cationic competition (Ca, Mg and Na) and dissolved organic matter (DOM) was assessed with the goal of developing toxicity prediction models. Standard methods (Environment Canada) were used for culture and testing which was done in intermediate hardness waters (60 mg/L $CaCO_3$, pH 7.2, Ca 0.5 mM, Mg 0.15 mM). With some exceptions, Ca and DOM provide protection against toxicity but the incorporation of these effects into toxicity prediction models was inhibited by a lack of understanding of solution geochemistry. The pH of test solutions had a significant influence on toxicity with, for example, low pH resulting reduced Dy toxicity.
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Pharmaceuticals in the Environment – Potential Environmental and Human Health Impacts

526 Pharmaceuticals in Nigerian waters: An emerging pollutant

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The levels and occurrence of pharmaceuticals in waters across the globe is on the increase and there are concerns that if not given quick attention it might as well be a threat to human and other animals who depend on it for survival. The situation is not different in Nigeria, as there are reports on the presence of active pharmaceutical substances in surface water and underground water in high industrial communities and non-industrial communities. The sources of pharmaceutical contaminants in Nigeria waters include hospitals waste and agricultural waste due to veterinary use and livestock feed additives. Pharmaceuticals that have been detected in water have been reported to induce reproductive and developmental effects such as decreased fertility, cancers, and other diseases in humans, wildlife, and laboratory animals. In few instances where pharmaceuticals have been measured in Nigerian surface waters (Chloramphenicol, Diclofenac, Erythromycin, and Sulfamethoxazole), were found at concentrations exceeding ecotoxicological effect concentrations, a situation that calls for serious health concern. The Nigerian Government should encourage its agencies to continue working together with the researchers in tertiary institutions and other partners to remain abreast of the latest developments in water quality, treatment, and public communication in establishing monitoring programs for emerging contaminants, especially pharmaceuticals in our water bodies.

527 Understanding the fate of chemicals in land applied materials using multi-scale field studies

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Contaminants of emerging concern (including pharmaceuticals) are often reported in aquatic monitoring studies. A direct pathway into the environment is via discharge into rivers, if not fully removed during wastewater treatment. However, for some substances, a large fraction may be removed in the wastewater treatment process in the form of sludge. An additional pathway can occur when the sludge is land-applied as biosolids, with movement to surface water if overland runoff or erosion occurs. To understand the potential environmental exposure resulting from runoff or erosion of biosolids, field scale runoff studies real-world provide exposure data. The direct measurement of runoff and erosion under controlled field settings can be used to inform exposure modeling, to explore mitigation evaluation, and ultimately refine estimated environmental concentration calculations. Multi-plot small-scale runoff studies (ft^2) can rapidly test multiple application and vegetation scenarios under simulated rainfall. These studies can also integrate a variety of soil and slope conditions. Larger landscape scale runoff studies (ft^2 to acres) assess greater variability and may incorporate subunit environmental fate investigations. Studies at this larger scale are designed to utilize simulated or natural rainfall. Both small- and large-scale study designs produce total and flow dependent mass loading data to assess the fraction of applied chemical which is transported under defined conditions. Watershed scale runoff studies (acres to mi^2) are designed to evaluate broader land use and the effect on surface water quality. Stream loading, hydrologic, and land use data are generated to fully understand the impacts that temporally or spatially distributed environmental variables may have on results. The time scale for these monitoring studies span from sub-day to multi-year. Although runoff studies conducted under USEPA Good Laboratory Practice Standards have been used for many years to support pesticide risk assessment, these types of studies can be readily applied to measure transport and fate of any land applied chemical for ultimate use in environmental risk assessment.

529 Screening Level Values for Pharmaceuticals in Water: Providing Human Health Risk Context to Environmental Detections

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Pharmaceuticals are increasingly looked for and detected in the environment. They are formulated for maximum potency and may have adverse as well as therapeutic effects at low levels. As a result, their presence in potential drinking water sources has raised concerns because of the risk to human health that these substances may pose. To help address this issue, the Minnesota Department of Health (MDH) developed a method for deriving screening level values for pharmaceuticals found in water. The developed methodology relies on existing drug label information, and uses the lowest therapeutic dose (LTD) as the point of departure (POD) with the application of both typical and non-traditional uncertainty and adjustment factors modified for pharmaceuticals. The resulting values are designed to be conservative (i.e., can be consumed daily with no anticipated health risk to humans) and are intended for use in prioritization and screening efforts. Using the developed methodology, MDH has developed water screening values for 119 pharmaceuticals that are commonly prescribed and/or commonly included in monitoring schedules used in Minnesota. Examples of drug classes included are antibiotics, antidepressants and anxiolytics, steroids, and others. MDH has used the derived screening values to provide risk context to pharmaceutical monitoring results for Minnesota. The majority of the pharmaceuticals analyzed for and detected in Minnesota waters have been found at levels below the developed screening values. In general, these values can be used to provide context for the potential human health risk of environmental detections, help prioritize future monitoring and analytical development, and to assist in evaluating general water quality.

530 Transpiration strongly impacts plant uptake of pharmaceuticals: Experiments and modeling

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In arid and semi-arid regions, reclaimed wastewater is frequently used for irrigation, resulting in crop plant exposure to contaminants that are commonly found in wastewater treatment effluents, such as pharmaceuticals. Previous studies show that uptake of pharmaceuticals into edible plant tissues is possible under field conditions. However, our understanding of pharmaceutical movement into and through plants contains many gaps, especially for ionizable compounds, which are affected by the surface charges and electrochemical gradients across membranes in addition to the hydrophobic interactions that govern the behavior of uncharged organic molecules. Previous attempts at modeling plant accumulation of ionizable compounds based on pK_a values and octanol-water partitioning coefficients has met with limited success. We tested a compound specific, transpiration-based model for plant accumulation of several pharmaceuticals using our own data, and found that transpiration accounts for much of the variation in accumulation among individual plants. We grew hydroponic spinach plants and exposed them to a variety of pharmaceuticals via their roots. We measured compound loss from solution and accumulation in plant roots and above-ground tissues, as well as transpiration, temperature, humidity, and light intensity. We determined that the extent of accumulation depends on the compound and does not easily correlate with commonly reported compound physicochemical properties. We also compared our results to accumulation predicted by an existing model for plant uptake of ionizable organic contaminants that accounts for electrochemical gradients between compartments in the plant. This work improves understanding of the parameters necessary for accurate prediction of crop plant uptake of ionizable organic contaminants.

531 Assessing Ecological Risk from the use of a Veterinary Medicinal Product in Cattle: Innovative Approaches for Wildlife Exposure Assessment

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Regulatory approval of veterinary medicinal products (VMPs) requires an assessment of the potential exposures to and effects on ecological receptors for these compounds in the environment. Banamine Transdermal Pour-On for Beef and Dairy Cattle contains the active pharmaceutical ingredient (API) flunixin, which is a non-steroidal anti-inflammatory drug (NSAID). Avian receptors, in particular old world vultures, have been shown to be vulnerable to related APIs (namely diclofenac) after feeding on tissue from treated livestock. Furthermore, specific routes of exposures for birds to pesticides topically applied to livestock have been identified. Impacts to magpies after consumption of hair from cattle treated with the organophosphate famphur and secondary poisoning of raptors consuming affected magpies have been reported. These two factors – potential effects of NSAIDs on birds and previously observed avian exposure to pesticides through cattle hair ingestion – triggered a focus on assessing risks to avian receptors, in addition to mammalian receptors, in the environmental assessment (EA) for Banamine Transdermal. The following exposure pathways were evaluated: predatory and scavenging birds and mammals consuming tissues from treated cattle, birds that may perch on the backs of cattle and ingest treated hair, and secondary poisoning of predatory and scavenging birds and mammals (i.e., via consumption of birds that have ingested treated cattle hair). Available guidance for conducting EAs for VMPs does not address these exposure pathways; thus innovative approaches were developed, including the experimental determination of the amount of cattle hair that can fit into the magpie gizzard. Results from this assessment demonstrated that the use of Banamine Transdermal is not expected to cause significant adverse impacts to avian or mammalian receptors. The environmental assessment supported a Finding of No Significant Impact by the U.S. Food and Drug Administration from the use of Banamine Transdermal in beef and dairy cattle in the US.

532 Environmental assessment of a veterinary drug (Revalor-XR) containing trenbolone acetate and 17β-estradiol

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Environmental assessments of pharmaceuticals are required by regulatory authorities as part of the drug approval process. Revalor-XR is an extended-release implant for use in steers and heifers that contains trenbolone acetate and 17β-estradiol (17β-E2) as active pharmaceutical ingredients (APIs). Both APIs are metabolized in situ resulting in the excretion of 17β-trenbolone (17β-TB), 17α-trenbolone (17α-TB), trendione (TBO), 17β-E2, 17α-estradiol (17α-E2), and estrone (E1). The similarity in chemical structures and many of the environmental fate properties among 17β-TB, 17α-TB, and TBO, and that among 17β -E2, 17α-E2, and E1 promote the use of surrogate compounds to represent the trenbolone compounds and the estradiol compounds in the environmental assessment. Data on the individual compounds were collected from various laboratory studies, aggregated to generate representative values for the surrogate compounds to characterize their environmental fate, and used for exposure assessments at feedlot-, field- and watershed-scales using USEPA's EXPRESS and BASINS models. Nine exposure pathways were evaluated at the feedlot and field scale, allowing for elimination of insignificant pathways for the watershed-scale modeling, which considered the major exposure pathways and was conducted for two representative watersheds, one in Texas and one in Iowa. The outputs of the modeling efforts resulted in Predicted Effect Concentrations (PECs) for individual as well as aggregated exposure pathways. The effects assessment was focused on potential reproductive impacts to fish from chronic exposure, which is the most sensitive ecological endpoint for these compounds, and generated Predicted No-Effect Concentrations (PNECs) for 17α-TB, 17β-TB, 17α-E2, and 17β-E2. Risk characterization involved comparison of the PECs for the surrogate compounds to the PNECs of the individual compounds, conservatively using the higher toxicity of the β-isomer in calculation of the risk quotients. The assessment at the watershed scale demonstrated that it is highly unlikely that the compounds associated with Revalor-XR would have any significant environmental impacts when used according to the Revalor-XR label. The environmental assessment supported a Finding of No Significant Impact by the U.S. Food and Drug Administration from the use of Revalor-XR in beef steers and heifers in the US.

533 Determining potential adverse effects in marine fish exposed to pharmaceuticals with the fish plasma model and whole-body tissue concentrations

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The Fish Plasma Model (FPM) was applied to water exposure and tissue concentrations in fish collected from two wastewater treatment plant impacted estuarine sites. In this study we compared predicted fish plasma concentrations to Cmax values for humans, which represents the maximum plasma concentration for the minimum therapeutic dose. The results of this study show that predictions of plasma concentrations for a variety of pharmaceutical and personal care products (PPCPs) from effluent concentrations resulted in 37 compounds (54%) exceeding the response ratio (RR = Fish [Plasma] / 1%$Cmax_{total}$) of 1 suggesting the potential for adverse physiological effects compared to 3 compounds (14%) detected with values generated with estuarine water concentrations. When plasma concentrations modeled from observed whole-body tissue residues were used, 16 compounds out of 24 detected for Chinook (67%) and 7 of 14 (50%) for sculpin resulted in an RR_{tissue} value greater than 1, which highlights the importance of this dose metric over that using estuarine water. Because the tissue residue approach resulted in a high percentage of compounds with calculated response ratios exceeding a value of unity, we believe this is a more accurate representation for exposure in the field. Predicting plasma concentrations from tissue residues improves our ability to assess the potential for adverse effects in fish because exposure from all sources is captured. Tissue residues are also more likely to represent steady-state conditions compared to those from water exposure because of the inherent reduction in variability usually observed for field data and the time course for bioaccumulation.

Oil and Gas Waters – Integrating Analytical Chemistry and Toxicology to Inform Management Decisions

534 Environmental NGO Perspective on Risks and Research Needs for Alternative Management of Produced Water

N. Saunders, C. Danforth, Environmental Defense Fund / US Climate & Energy

Facing disposal complexities from seismicity, drought, and calls for conservation, alternative management strategies for part of the nearly 900 billion gallons of wastewater produced each year by the oil and gas industry are being implemented or considered across the country – yet not enough is known regarding the risks to human health and the environment from these practices. It is vital for researchers and regulators to identify and fill knowledge gaps surrounding produced water and proposed uses outside the oil-field before practices are implemented that may create more risks than they resolve. This presentation will include a representation of research and risk management gaps and needs alongside learnings from expert workshops on the subjects of produced water chemical characterization, treatment technology, and toxicity assessment. An overview of ongoing, collaborative scientific research with universities across the country on these subjects will also be presented, including but not limited to, analytical methods for the identification and quantification of organic chemicals, biological treatment technologies, and application of novel bioassays for toxicity assessment of produced water. Numerous collaborators are expected to present in more depth separately throughout the meeting.

535 Effluent Characterization of Treated Produced Water from Unconventional Oil and Gas Production

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The Eureka Resources, LLC (Eureka) Standing Stone centralized waste water treatment facility receives produced and flow back water from unconventional oil and gas operators in the Marcellus Shale play and treats the water before being discharged to surface water under an NPDES discharge permit. Shell is one of the operators whose flow back and produced water is treated at the Standing Stone facility and has worked with Eureka and the Center for Responsible Shale Development (CRSD) to assess the monitoring data from the facility effluent. Substantial chemical characterization and Whole Effluent Toxicity (WET) testing of the effluent has been performed beyond NPDES monitoring requirements to assess the effluent hazard and risk to the receiving water. Based on the monitoring results, no impact on the receiving environment is expected and this disposal route has been recognized by CRSD as a sustainable option for produced / flow back water management. An overview of the treatment process(es) will be presented. The effluent chemistry and WET testing data will be summarized along with how the data were assessed. Data will also be presented from Eureka's Williamsport 2nd Street facility, which has an indirect discharge permit allowing discharge of condensate from an evaporator to be discharged to a local POTW. Although the facility has not been reviewed by CRSD, it meets all regulatory requirements and produces a condensate effluent that is treatable by the POTW.

536 Long term marine fate and effects of Enhanced oil recovery polymers - A analytical challange

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The Norwegian offshore adventure is years beyond peak oil. And while the green change is well underway, for example having the largest per capita use of electric cars, the economy will still depend on petroleum extraction for years to come. The government has together with big oil invested in a national increased oil recovery research center in order to smoothen the transition from a black to green economy. The aim is to develop sustainable offshore increased oil recovery methods. A key technology being researched is polymer flooding. Polymer flooding is largely proven to be a viable method for increasing oil recovery rates. Albeit, polymer flooding also has the potential to become an unprecedented source of water-soluble synthetic linear high molecular weight polymers in the ocean. Worryingly, there is a general lack of understanding regarding these polymers long-term marine fate and effect, other than having apparent little biological activity. In addition, developing methods for monitoring and characterizating polymers is critical for produced water management purposes. In this respect, accurately measuring the molecular weight distribution change over time is of particular interest as an absolute measure of degradation. With the advent of commercial light scattering technology, it is now be possible to address these issues better than ever before. Thus far, various polyacrylamide-based polymers has been exposed to North sea-like marine conditions for an extended period of time as a part of a standardized static respirometry test. Now the challenge remains to isolate and quantify the structural/chemical changes through the use of size exclusion chromatography coupled with static multi angle laser light scattering and a few other complementary assays. In turn, the data generated will be used to establish structure activity relationships that can be used for environmental risk assessments and to select the best performing polymer. We will present the results of the first full scale study at the Twin cities SETAC 38th annual meeting.

537 Toxicity of produced water from offshore oil production in Norway and corresponding polar and apolar fractions

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Regulation of produced water (PW) discharges on the Norwegian continental shelf is based on a maximum oil in water limit of 30 mg L-1. The use of conventional oil quantification methods based on traditional GC is limited when it comes to polar compounds which may originate from both the produced crude oils and the use of treatment or production chemicals, might still contribute to the toxicity of the PW. Thus, for PWs there might therefore be discrepancies between the measured concentrations of organic compounds, and the total amount of compounds that may contribute to toxicity. PW was collected from the "point of release" on four oil platforms on the Norwegian continental shelf. The PWs were from oil fields of different operational ages, and the oils produced exhibited different physical and chemical properties. Samples were subjected to extraction with dichloromethane, followed by fractionation into apolar and more polar fractions using solid phase extraction, recovering 80 % of the total GC amenable material in these fractions. Fractions and total extracts were thoroughly characterized using GC-MS, GCxGC-MS, LC-Orbitrap MS, and by direct infusion FT-ICR MS. The total PW extract, as well as the polar and apolar fractions were subject to acute toxicity tests using Acartia tonsa nauplii. The LC50 values for the total PW extracts ranged between 0.05-0.98 mg L-1 (based on total GC amenable fraction analysis). For three of the PWs, the toxicity was mainly attributed to the polar fractions, with LC50s of 0.17-0.57 mg L-1. Interesting, for the fourth PW the toxicity was mainly attributed to the apolar fraction, with a LC50 of 0.05 mg L-1. For the PWs with toxicity mostly related with the polar fraction, this fraction spanned from 16-55% of the total PW (GC amenable fraction analysis), and for the PW with toxicity mainly in the apolar this was 35%. This study demonstrates that PW toxicity may be associated with compounds that are currently poorly characterized. Polar fractions, in particular, may contain compounds not amenable by GC, or even contributing to the GC- based quantification of oil in water. This suggests that PW toxicity is not directly correlated with the GC quantifiable compounds that are used for regulating discharges today. Further studies should be pursued with a wider array of PWs from a range of sources to determine if alternative methods of characterization are needed for regulation of PW discharges.

538 Making Cumulative Risk Assessment of Offshore Produced Water Discharges Tractable

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Chemical analysis and toxicity testing using six marine test species was used to characterize the hazard of produced waters from twelve Australian offhsore platforms. Using these site data and platform specific plume discharge and species sensitivity distribution modeling, cumulative risks were quantified by calculating the multiple stessor potentially affected fraction (msPAF) of the species in the local marine environment. Results provided two independent lines of evidence that cumulative risks to marine life from these discharges are low and acceptable with intended 95% protection goals. A limited number of produced water constituents (hydrocarbons, sulfide, ammonia) were found to dictate risk thereby providing a rationale for more targeted analysis in future monitoring studies. Based on these findings a tiered framework to foster consistent screening and potential refinement of cumulative risk evaluations of produced water discharges is proposed.

539 In Vitro Assessment of Endocrine Disrupting Potential of Organic Fractions extracted from Hydraulic Fracturing Flowback and Produced Water

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There is increasing public concern on the potential hazards regarding the leak or spill of hydraulic fracturing flowback and produced water (HF-FPW) into the aquatic environments. HF-FPW is a waste water returned to the surface after well stimulation activity, which contains a large amount of salts, metals, natural organics, anthropogenic additives, and potential secondary byproducts from downhole reaction. While surface spills of HF-FPW are known to occur, the potential hazards of this wastewater on the health of aquatic ecosystem have not been fully characterized. We are the first research group investigating the toxicological impact of these wastewaters to various aquatic models, including water flea (*Daphnia m.*), zebrafish, and rainbow trout. Previous studies indicated HF-FPW can significantly disrupt the regulation of biotransformation and endocrine gene expression as well as reproduction in aquatic species. However, the mechanism of these endocrine disruptive effects and the temporal and spatial variation of HF-FPW samples on toxicity were still not fully understood. In the current study, 12 organic extracts were isolated from HF-FPW samples collected from 2 different sites with 6 timepoints. PAH contents and total organic profiles were also analyzed by GC-MS and HPLC-Orbitrap. Luciferase reporter gene assays using H4IIE-luc, MDA-kb2, and MVLN-luc cell lines were used to screen the agonistic and/or antagonistic activities against aryl hydrocarbon receptor (AhR), androgen receptor (AR) and estrogen receptor (ER), respectively. The results demonstrated the complexity of receptor mediated signaling effects of organic fractions of HF-FPW, especially on induction of AhR activity. The current study also investigated the endocrine disruptive properties of HF-FPW samples collected from different locations and time points, suggesting that the organic contaminants released from HF-FPW spills may have various endocrine disrupting potentials on aquatic organism.

540 The toxicity of chloride to duckweed and Daphnia in reconstituted and natural waters with high calcium hardness and total dissolved solids

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Large volumes of co-produced waters are a byproduct of both conventional and unconventional oil production. Produced water volumes can exceed 10X oil production over the lifespan of a well. Production water is often highly saline, with total dissolved solid (TDS) concentrations over 350,000 mg/L. In the Williston Basin, North Dakota, produced waters are dominated by Cl, with concentrations of over 200,000 mg Cl/L. The composition of background surface waters in the region is highly variable, but these waters are generally characterized by high hardness and TDS, but relatively low concentrations of Cl. Recent work has revealed potential ameliorating effects of calcium hardness on toxicity of Cl to aquatic organisms, but little research has been conducted to evaluate the toxicity of Cl in naturally hard, complex mixtures. We performed bioassays to evaluate the toxicity of Cl to growth of duckweed (*Lemma gibba*) and survival of the cladoceran, *Daphnia magna* in high TDS, high hardness reconstituted waters simulating surface waters of the Williston Basin. We also evaluated the growth of duckweed in water collected from reference and contaminated sites from the Goose Lake National Wildlife Refuge in NE Montana. The 7-day acute EC-50 of Cl in reconstituted water was 2,931 mg Cl/L, and 21-d EC-50 was 1,620 mg Cl/L in the chronic exposure. The 48-hour LC-50 for *D. magna* was 3,788 mg Cl/L. In water collected from Goose Lake, yellowing and withering of duckweed fronds and a complete lack of growth was observed in all exposures at all contaminated sites. At the least contaminated site (5,650 mg Cl/L), significant effects on growth were observed at 100%, 75% and 50% site water diluted with reference waters, and the 7- day EC-50 was 2,645 mg Cl/L.

541 Toxicity analysis and effects on zebrafish swim performance of hydraulic fracturing flowback and produced water as a function of flowback time

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Horizontal hydraulic fracturing processes involve pumping water, proppants, and fracturing chemical additives into the sub-surface at high pressures to recover gas and oil from geological shale formations. To date, little toxicological information is supplied for the wastewater which flows after well pressure has been released, termed flowback and produced water (FPW). Many inorganic (e.g. metals, salts etc) and organic (e.g. polyaromatic hydrocarbons, etc.) chemicals are found in FPW which differentially change in concentration depending on the time they are collected from the well bore. Correspondingly, toxic potential of this fluid may similarly change pending on retrieval time. Lethality in multiple model organisms (zebrafish embryos, rainbow trout, and *Daphnia*) to three temporally different FPW samples collected from one horizontally fractured well was analyzed. LC50 analyses over 96 hours were performed on 3 temporally different samples to determine if there is a change in toxicity related to exposure of FPW. Furthermore, inorganic and organic water chemistry characterization was performed on each FPW sample to determine if changes in FPW toxicity are associated to changes in chemical concentration profiles. To determine if embryonic exposures to one FPW sample induced sub-lethal effects which persisted to later life stages, we also measured swim performance (U_{crit} measured in body lengths/s) and swimming metabolic capacity (factorial aerobic scope; F-AS) of juvenile zebrafish (60 dpf exposed as embryos for 24 or 48 h) was analyzed. FPW exposure significantly decreased both juvenile fish U_{crit} and F-AS values following acute embryonic sub-lethal exposures. Our results aim to provide industry and regulatory agencies information on FPW toxicity and how to effectively minimize risk to the environment should FPW releases to the surface waters occur. Additionally, our research is the first to show impacts on fish fitness following embryonic FPW exposures.

Wildlife Ecotoxicology – Linking Exposure to Effect Cascades

542 Histopathology and lung assay as potential biomarkers of chronic exposure in rodents at uranium mine sites

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The effects of chemical constituent exposure to biota at breccia pipe uranium mine sites in the Grand Canyon watershed (Arizona, USA) have not been studied. The U.S. Geological Survey is conducting studies at mining (active, recently active, and closed status) and reference (no mining activity) locations to determine if uranium ore-related minerals are associated with chemical burdens and pathologies in rodent tissues. This study focuses on analytical chemistry, histopathology, and lung assay results for deer mice (*Peromyscus* spp.), cliff chipmunk (*Tamias dorsalis*), and Valley pocket gopher (*Thomomys bottae*) from reference and mining sites. We examined logistic regression between inorganic contaminant concentrations (e.g. uranium, arsenic, cadmium, copper) and the presence of histological anomalies in the liver, kidney, and lungs. The most common anomalies identified were glycogen vacuolation, mineralization, and extramedullary hematopoiesis in the liver; mineralization and regeneration in the kidney; and inflammation and increased numbers of macrophages in the lung. Several of these anomalies were significantly associated with a variety of chemical concentrations in the whole-body or kidney tissues in mice. These results may indicate anomalies were general, not contaminant-specific, health indicators. We also examined if weathering of mining material influences the inhaled particulate composition, which could result in differences in mineral deposition and pathology present in the lung. Lung tissue from animals collected at reference and mining sites were examined with scanning electron microscopy and energy dispersive spectroscopy to determine the size and composition of particulate matter in areas with inflammation, bronchiolar hyperplasia, mineralization, intracellular refractile debris, macrophages, and parasitic pathology. Particulate matter, generally less than 1 micrometer, observed in tissues included titanium dioxide, iron oxide, calcite, Al-Si fly ash particles, clay, feldspar, and zircon. Small sample size limited any comparisons between mined and reference specimens in type or size of particulate matter and in tissue pathologies to untestable observations.

543 Assessing Toxicogenomic Effects of 17β-Trenbolone on the Japanese Quail Reproductive Axis

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Trenbolone-acetate is a synthetic anabolic steroid used as a livestock growth promoter. The breakdown product, 17βtrenbolone (17βT), is a known endocrine disruptor; elevated concentrations of 17βT have been reported in solid dung collected from livestock farms. Despite concerns over the endocrine disrupting potential of 17βT in aquatic and avian species, to date, only a limited number of studies have studied 17βT in birds, examining sub-lethal effects on the reproductive organs of Japanese quail (JQ) along the hypothalamic-pituitary-gonadal-liver (HPGL) axis. These studies, conducted at the USGS, reported reduced numbers of maturing yolk follicles, lowered egg production, decreased plasma testosterone concentrations and changes in mRNA expression of relevant genes such as vitellogenin (VTG). However, they examined one time point, late in egg laying, after 13 weeks of exposure. We suggest that by looking at a point soon after sexual maturation, we may be able to determine whether the bird compensates for the exogenous endocrine stress, or whether the biochemical/genomic response is inadequate, thereby resulting in adverse effects on reproduction. Additionally, there is the need to obtain a better understanding of which changes along the HPGL axis could be specifically linked to changes in egg production. The objective of our research was to build upon previous studies, exposing 7 week old JQ to 17βT (0, 5 or 20 ppm) in their diet, and collect samples on days 3, 7, 14 and 21 of exposure, allowing us to focus on earlier exposure windows. Plasma collected from the birds were analyzed for reproductive hormones and Zn concentration (which is a commonly used surrogate for plasma VTG levels). Liver and gonadal tissues were analyzed for changes in mRNA expression of key genes along the reproductive axis such as VTG, ApoVLDL, estrogen receptors (α and β), androgen receptor, 17β hydroxysteroid dehydrogenase and aromatase. No significant differences in gene expression were found in male liver tissues. Morphometric analyses showed a decrease in the female hepatosomatic index (HSI) and total follicle weight after 21 days of exposure, in the 20 ppm dose. Female plasma Zn concentration was also significantly decreased after 21 days of exposure in the high dosed group. Results from the female liver and ovary gene expression and hormone analyses could provide more mechanistic information on the observed morphometric and plasma Zn concentration differences.

544 Linking sublethal effects of 17β-trenbolone to adverse reproductive outcomes in Japanese quail

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There have been recent initiatives to model the physiological processes involved with egg production and growth in numerous taxa, but to date, there are no such avian models. These models have great utility for determining how perturbations to physiological systems could enhance or inhibit reproduction or growth. Environmental contaminants can cause adverse reproductive effects in vertebrates through the disruption of the hypothalamic-pituitary-gonadal-liver (HPGL) axis. Trenbolone-acetate is a synthetic anabolic steroid used as a livestock growth promoter and its metabolite, 17β-trenbolone, has been shown to disrupt key physiological processes related to the endocrine system including egg production in birds. In order to parameterize a computational avian vitellogenesis model, we investigated the effects of a three-week exposure to 17β-trenbolone on sexually maturing female Japanese quail (*Coturnix japonica*). Egg production was recorded daily and hormones along the HPGL axis were quantified at four time points after the start of exposure. Early results indicate that exposure to 17β-trenbolone reduced vitellogenin, yolk and egg production. This work aims to establish the first avian vitellogenesis model providing researchers a tool to estimate the number of eggs produced by an individual bird and help estimate the adverse effects of contaminant exposure on avian reproduction.

545 Hepatic transcriptomic profiling of double-crested cormorants collected from variably contaminated breeding colonies of the Great Lakes

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Determining the effects of complex mixtures of environmental contaminants poses many challenges within the field of ecotoxicology. Previous studies from our lab evaluated the effects of complex organic extracts derived from avian eggs collected from variably contaminated breeding colonies on gene expression in cultured chicken hepatocytes. The goal of these studies was to link geographically distinct contaminant profiles with unique transcriptomic signatures. In this study, we further explore the utility of transcriptomic profiling by comparing gene expression patterns in liver tissue of double-crested cormorant (DCCO; *Phalacrocorax auritus*) embryos collected from six Great Lakes breeding colonies, and a non-Great Lakes reference site. Eggs collected from the breeding colonies have variable organohalogen contaminant concentrations and therefore we attempt to identify gene expression patterns associated with the colony of origin. Hepatic transcriptomic profiles of nine individual, late-stage embryos from each breeding colony were assessed using a custom-designed DCCO ToxChip polymerase chain reaction (PCR) array comprising 27 target genes associated with several toxicologically-relevant pathways (e.g. xenobiotic metabolism, thyroid hormone pathway, lipid homeostasis, immune function). Heat-map clustering of mRNA expression data permitted the spatial organization of DCCO colonies with regards to modulation of toxicologically-relevant pathways. The application of transcriptomics to a wild avian species, naturally exposed to a complex mixture of chemicals and other stressors, represents a means to detect subtle perturbations that ultimately could be linked to adverse outcomes at the individual level.

546 Toxics as a limited factor for killer whale recovery: Persistent organic pollutants and PAHs in killer whale scat samples

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The Southern Resident killer whale population (*Orcinus orca*) was listed as endangered in 2005 and shows little sign of recovery. These fish eating whales feed primarily on endangered Chinook salmon. This study demonstrates population growth is constrained by diminishing prey abundance, excessive exposure to toxicants, and low offspring production using scat samples collected between 2008-2014. Exposure to persistent organic pollutants (POPs) is associated with adverse health effects including endocrine disruption and reproductive toxicity. The scat samples from this study demonstrate that POP levels are highest, from endogenous lipid stores, and are comprised of more toxic compounds when the whales primary prey source is at low seasonal abundance. Accumulation patterns showed an expected age-related increase, excepting nulliparous females that may have toxicant offloading from unrecorded fetal or neonate loss. Additionally measures of polycyclic aromatic hydrocarbon were performed in the scat samples providing baseline data in the circumstance of an oil spill event and subsequent exposure in the inland waters of Washington state. This study also validated and applied temporal measures of progesterone and testosterone metabolites to assess occurrence, stage, and health of pregnancy from genotyped killer whale feces. Thyroid and glucocorticoid hormone metabolites were measured from these same samples to assess physiological stress. These methods enabled us to assess pregnancy occurrence and failure as well as how pregnancy success was temporally impacted by nutritional and other stressors. Up to 69% of all detectable pregnancies were unsuccessful; almost half of these failed relatively late in gestation or immediately post-partum, when the cost is especially high. Low availability of Chinook salmon appears to be associated with late pregnancy failure, including unobserved perinatal loss. Release of lipophilic toxicants during fat metabolism in the nutritionally deprived animals may contribute to these cumulative effects. Results point to the importance of promoting Chinook salmon recovery to enhance population growth of Southern Resident killer whales. The physiological measures used in this study can also be used to monitor the success of actions aimed at promoting adaptive management of this important apex predator to the Pacific Northwest.

547 River Otters (Lontra canadensis) trapped in a coastal environment contaminated with POPs: Demographic and physiological consequences

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Productive coastal and estuarine habitats can be degraded by contaminants including persistent organic pollutants (POPs) such as PCBs, dioxins, and organochlorine insecticides to the extent of regulatory designation as contaminated sites. Top-predatory wildlife may continue to use such sites as the habitat often appears suitable, and bioaccumulate POPs and other contaminants with potential consequences on health and fitness. Victoria and Esquimalt Harbours are located on southern Vancouver Island, British Columbia (BC) and are federally designated contaminated sites due mainly to past heavy industrial activity. We collected fecal samples from river otter (*Lontra Canadensis*) throughout an annual cycle, and combined chemical analysis with DNA genotyping, and radio telemetry of a subsample of animals to examine whether the harbor areas constituted an ecological trap for otters. About 20% of individual otters exceeded published reproductive effect levels based mainly on data for mink (*Neovison vison)*, and there were significant correlations between concentrations of PCBs in fecal samples and of thyroid and sex hormones. However, analysis and modeling of the DNA genotyping data showed no directional movement away from the harbours, and thus no evidence that the harbor otters formed a sink population and therefore were in an ecological trap. Our study demonstrates the value of combining chemical and biological technologies with ecological theory to investigate practical conservation problems.

548 Trophic magnification of POPs within a terrestrial food-web of an avian top predator, the Cooper's hawk (Accipiter Cooperii)

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Several types of POPs, such as PCBs and DDE, flame retardants and perfluorinated compounds, are released from multiple sources into the ambient environment and can negatively impact many endocrine and physiological functions within exposed wildlife. Protocols to assess bioaccumulation of these persistent chemicals within terrestrial systems are far less developed compared to aquatic systems. Presently regulatory agencies in Canada, the USA, and the EU use only bioaccumulation information for fish to assess the bioaccumulation potential of chemicals. However, recent studies have shown that some chemicals that are not bioaccumulative in aquatic food-webs biomagnify in terrestrial food-webs. To better understand the bioaccumulation behavior of chemicals in terrestrial food-webs, we aim to produce a food-web model to assess the presence of and biomagnification of POPs in an apex avian predator, the Cooper's hawk. We focused our study sites around 17 Cooper's hawk nests within urbanized areas of Metro Vancouver. We sampled the hawk's food chain by collecting samples of common prey species, mainly American robins, European starlings, and House sparrows. To represent the lower trophic levels several species of ground-beetles, sow and pill bugs, earthworms, and Himalayan blackberries were collected. Soil and air samples were also collected near hawk nesting sites. All samples were analyzed for a number of contaminants listed as priorities for monitoring by the Chemical Management Plan of the Canadian federal government. Resulting data will be inputted into a food-web model to examine bioaccumulation processes in a terrestrial system. For instance, preliminary results reveal that perfluorinated compounds, specifically PFOS, significantly biomagnifies in the Cooper's hawk food-web with an estimated Trophic Magnification Factor of 4.37.

549 Contaminant Exposure and Productivity of Ospreys (Pandion haliaetus) Nesting in the Delaware Estuary and Coastal Inland Bays

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In 2015, ospreys nesting in Delaware River, Delaware Bay and the coastal Inland Bays of Delaware were monitored to examine spatial and historic trends in contaminant exposure and productivity. Osprey nests were visited at 7 to 10 day intervals. A sample egg was collected from 9 nests in each of these 3 study regions, and its contents were quantified for persistent, bioaccumulative and toxic contaminants. Blood samples were collected from 40-45 day old nestlings to assess exposure to pharmaceuticals, and to measure oxidative DNA damage (8-hydroxy-2'-deoxyguanosine) as a biomarker of genotoxicity. Concentrations of legacy organochlorine pesticides and metabolites, total polychlorinated biphenyls (PCBs) and polybrominated diphenyl ether flame retardants in eggs were greatest in Delaware River, but clearly lower in all regions compared to values reported in our 2002 study (Toschik et al. ET&C 24:617, 2005). A suite of alternate brominated flame retardants (TBPH, TBB, BTBPE, DBDPE) were rarely detected, and when present were at low ng/g quantities. A subset of eggs was analyzed for coplanar PCBs, and revealed a north (greatest) to south spatial gradient in concentrations and toxic equivalents. Of the 29 nestling plasma samples, only 2 of 21 pharmaceuticals exceeded detection limits (acetaminophen in 22 samples, diclofenac in 2 samples), and levels were typically 2 to 3 orders of magnitude below human therapeutic concentrations (Bean et al. 7th SETAC World Congress, Abst. 40, 2016). There was no evidence of eggshell thinning or regional differences in nestling blood oxidative DNA damage. The percentage of eggs lost, eggs hatched and young fledged did not differ among study regions. Compared to 2002, osprey productivity was slightly lower in the central region (1.11 fledglings/active nest in 2015 vs 1.42 in 2002) and in the coastal Inland Bays (1.00 fledglings/active nest vs 1.17 in 2002), while productivity was slightly greater in the northern region (1.22 fledglings/active nest in 2015 vs 1.00 in 2002), but these subtle differences were not statistically significant. Overall, productivity was adequate to maintain a stable population (0.8-1.15 fledglings/active nest). Our assessments of contaminant exposure, sublethal measurement endpoints, and productivity do not indicate substantial ecotoxicological risk for ospreys at the individual or population level, and serve to document their recovery and resilience in the face of anthropogenic threats.

Emerging Environmental Chemistry – Trends, Transformations and Fate of Organic Environmental Contaminants – Part 1

550 Characterization of Organic Micropollutants in Ship Ballast Water

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Globalization of commerce has led to increased reliance on shipping, with approximately nine billion tonnes of cargo transported annually by sea. A byproduct of this extensive network of oceanic transport is that an estimated 3-10 billion tonnes of ballast water is discharged from vessels carrying water from other parts of the world yearly. Water taken onboard during ballasting operations is expected to resemble that of the port surface waters, the quality of which is dependent on local pollution conditions. Organic micropollutants may be introduced into ballast waters through contamination of port water and directly from shipboard operating procedures. These compounds are discharged during the voyage into open ocean waters or within another port during de-ballasting operations. Thus, the potential exists for introduction of micropollutants to pristine waters as well as exchange and transport among global ports. To understand the chemical micropollutant burden of ship ballast waters, we sampled ballast water and paired port water from ships in the USA, South Africa and Singapore. The water was characterized for water quality parameters as well as for the presence of polar organic compounds by both targeted and non-targeted liquid chromatography with high-resolution tandem mass spectrometry. The results of these analyses confirmed the presence of multiple wastewater- and stormwater-associated organic pollutants, such as nicotine, DEET and atrazine, within every tank sampled. The compounds detected with greatest relative intensity and frequency in ballast waters included polyethoxylated surfactants associated with industrial detergents, such as polyethylene glycols, lauryl alcohol ethoxylates and nonylphenol ethoxylates. It is likely these compound classes represent both land- and ship-derived sources of pollutants to ballast water. Further, ballast water was found to be chemically similar to port water when ballasting was performed within that port. The potential residual impacts of shipping activities to coastal and port water quality will be discussed in this presentation.

551 Pharmaceutical Conjugate Distribution in a Municipal Wastewater Treatment Plant

A. Brown, University of Manitoba / Chemistry; C.S. Wong, University of Winnipeg / Chemistry

Acetaminophen (ACM), propranolol (PRO), sulfamethoxazole (SMX), thyroxine (THY), and their respective major conjugates acetaminophen sulfate (ACM-Sul), 4-OH-propranolol sulfate (PRO-Sul), *N*-acetyl-sulfamethoxazole (N-Ace-SMX), sulfamethoxazole-β-glucuronide (SMX-Glc), and thyroxine glucuronide (THY-Glc) were measured in a pilot-scale experiment of the North End Winnipeg Water Pollution Treatment Plant in Winnipeg, Canada over 3 months to estimate the distribution of these compounds. Until now, levels and distributions of such conjugates were unknown in any natural or engineered water. All compounds were extracted by weak anion exchange solid phase extraction, and quantified simultaneously by ESI+ LC-MS/MS using a HSS T3 hydrophilic medium-density C_{18} column. In final effluent water, concentrations of all parent drugs were 0.32, 0.041, 0.38, 0.006 µg/L for ACM, PRO, SMX, and THY respectively. There was a marked difference in conjugate distribution dependent on class. ACM-Sul attenuated from 84 µg/L in the primary to < LOQ in subsequent treatment. PRO-Sul was persistent throughout treatment at similar levels to PRO (0.04 µg/L), thus doubling the mass load. SMX, N-Ace-SMX, and SMX-Glc all attenuated approximately 70% after primary treatment. THY-Glc was persistent throughout treatment at similar levels to THY (0.01 µg/L), thus doubling the mass load. PRO, SMX, and THY were all found associated with suspended solids and return activated, and waste activated sludge at similar levels (0.05- 0.1 0.01 mg/g solids dry weight) each. THY-Glc was the only conjugate found associated with suspended solids/ sludges which accounted for >99% of the total mass load into the Red River. Upon UV tertiary treatment there appeared to be no additional attenuation of any compound, whether parent or conjugate. This study indicates that conjugates are most likely being released into receiving waters, and that total inventories for these drugs are greater than previously reported. Moreover, in agreement with other literature, analysing suspended solids matters in terms of calculating a more accurate total mass load into surface waters receiving wastewater input.

552 Atmospheric OH-PCBs in the Metropolitan Chicago area

N.J. Herkert, The University of Iowa / Civil and Environmental Engineering; A.M. Awad, The University of Iowa / IIHR-Hydroscience and Engineering; K.C. Hornbuckle, University of Iowa / Civil and Environmental Engineering

It has been hypothesized that oxidation by a hydroxyl radical is an important process for the fate of PCB congeners in the environment. The atmospheric lifetimes of OH-PCBs are predicted to be short, functioning as a highly reactive intermediate in the degradation pathway of PCBs in the environment, which make them difficult to detect in environmental air samples. Here, we present the results of >50 environmental polyurethane equipped passive air samplers (PUF-PAS) collected in the metropolitan Chicago area in 2012 and 2013 for a suite of 72 OH-PCB congeners. The most frequently detected congener (6OH-PCB2) was detected in ~70% PUF-PAS samples. This congener was recently detected in air as a direct volatilization product of original legacy Aroclor contamination. In addition to the evidence of direct volatilization from legacy contamination, there is evidence of OH-PCBs existing as an atmospheric PCB breakdown product for two of the sampling sites in this study.

553 Current use pesticides in the atmosphere of the Great Lakes

S. Wang, Indiana University - Bloomington / School of Public and Environmental Affairs; A. Salamova, Indiana University Bloomington; M. Venier, Indiana University - Bloomington / SPEA; R.A. Hites, Indiana University / School of Public Environmental Affairs

The United States is one of the largest producers, consumers, and traders of pesticides in the world. In 2012, U.S. pesticide use accounted for approximately 23% of the total amount pesticides applied worldwide.

However, little is known about the occurrence of current-use pesticides (CUPs) in the atmosphere of the US. Here we investigated the spatial trends of 17 CUPs, including 1 fungicide, 3 insecticides, 4 herbicides, and 9 pyrethroids, in air (vapor and particle phases) at the five United States Integrated Atmospheric Deposition Network sites located in the Great Lakes basin. These samples were collected from January to December, 2014. In general, higher CUPs concentrations are observed at the urban sites in Chicago and Cleveland, while Eagle Harbor, the remote site, had the lowest concentrations of these contaminants. These results suggest that the spatial differences of CUPs usage might be the potential reason. Cyfluthrin, cypermethrin, and chlorothalonil are the most abundant CUPs in Chicago, Cleveland, and Sturgeon Point, averaging at 47, 23, and 147 pg/m^3, while esfenvalerate has the highest level at Sleeping Bear Dunes (average 82 pg/m^3). Higher levels of CUPs were observed in the particulate phase than in the vapor phase. The physicochemical properties of those CUPs, with high K_{oa} values, are the controlling factors of their partitioning between the vapor phase and the particulate phase.

554 Metabolites of Organophosphate Ester and FireMaster® Flame Retardants in Addled Bald Eagle Eggs from the Great Lakes Region

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Twenty two addled egg samples were chosen from the Michigan Bald Eagle Biosentinel Program archive, which were collected from bald eagle (Haliaeetus leucocephalus) nests within the state of Michigan between 2000 and 2012. Sampling sites can be divided into two groups: Inland (IN) and Great Lakes (GL) breeding areas. A suite of organophosphate esters (OPEs) (triaryl and trialkyl phosphates) and brominated FireMaster® flame retardants and their corresponding metabolites were targeted for analysis. Briefly, OPE and FireMaster analytes were extracted by soxhlet and cleaned in three stages; a Florisil® column, a Z-Sep/C18 sorbent mixture, and then a polymerically bonded aminopropyl phase cartridge. Concentrations of six OPEs in eagle eggs were generally low (geometric mean 24 ng/g ww) and the two main FireMaster components 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EHTBB) and bis(2-ethylhexyl) tetrabromophthalate (BEHTBP) were below limits of detection. This together with similarly low concentrations reported for egg and serum samples in birds from other studies, led to a hypothesis of potential rapid metabolic transformation of these compounds. A novel combination of refinement to previously published extraction and clean-up techniques, together with recently advanced LC-MS/MS methodologies, enabled the development of a new analytical method for both OPE and FireMaster metabolites in environmental biological matrices. Briefly, metabolites were extracted by accelerated solvent extraction using acidified acetonitrile. Extracts were then cleaned using an aminopropyl silica gel SPE column. The analysis of OPE metabolites and FireMaster® metabolites in environmental biological matrices is an emerging research area and relatively few research articles on this subject exist in the literature. Preliminary results on the analysis of metabolites in egg samples suggest that the most abundant OPE metabolites are bis(2-chloroethyl)phosphate (BCEP) and bis(2,3-dibromopropyl)phosphate (DBPP).

555 Contaminants of Emerging Concern (CECs) in Sport Fish from Russian River Watershed, California, USA

T.W. Jabusch, San Francisco Estuary Institute / Environmental Toxicology; R. Sutton, J.A. Davis, San Francisco Estuary Institute; W. Lao, Southern California Coastal Water Research Project; K.A. Maruya, SCCWRP; R. Fadness, California State Water Resources Control Board - North Coast Region

Polybrominated diphenyl ethers (PBDEs) and perfluorinated alkyl substances (PFASs) are typically not included in routine monitoring programs and specific water quality objectives have not yet been developed. These CECs accumulate in biota and may pose a threat to human and wildlife health. Important sources to surface waters include wastewater effluent and stormwater runoff. However, data about sources and the occurrence of CECs are generally lacking. The current study evaluated PBDE and PFASs concentrations in fish tissue samples collected from sites in the Russian River watershed. Results were evaluated in relationship to potential sources and pathways. The sums of PBDE congener concentrations (range: 0.1 – 30 ppb, median: 3 ppb) were lower than ranges observed in fish from San Francisco Bay and in Southern California coastal urban watersheds. Results indicate that PBDE concentrations in fish tissue samples collected from sites in the Russian River watershed were well below thresholds of concern for human consumption. Fish tissue levels of PFOS/PFASs also appear to be of limited concern, particularly for human consumption. PFOS was detected in all thirteen samples analyzed (range: 1 – 11 ppb) and the dominant PFAS. Along the Russian River, the highest PFOS concentrations for two examined species were in fish from the stations closest to the coast and farthest from upstream locations that are closer to expected stormwater and wastewater discharge points. Potential pathways may include discharges from tributaries entering the lower reach of the river and land application of biosolids for wastewater treatment plants.

556 Azobenzene disperse dyes in house dust: Emerging indoor environmental contaminants relevant to human exposure

L. Ferguson, Duke University / Nicholas School of the Environment / Civil and Environmental Engineering; H.M. Stapleton, Duke University / Nicholas School of the Environment

Disperse dyes are a class of substituted anthroquinone- or azobenzene-based dyes used to color synthetic fabrics such as polyester, nylon, and acrylic. They are high production-volume commodity chemicals and have been the fastest growing class of synthetic dyes since the 1970s. Although occurrence of a few disperse dyes have been reported in the aquatic environment previously, and the fate and reductive transformation of these compounds in aquatic sediments has been studied since the early 1990s, very little is known about their occurrence and fate in the indoor environment, where direct human exposure is a primary concern. Apart from their known mutagenicity, disperse dyes are also implicated as contact allergens, thus their occurrence and human exposure potential warrant detailed study. We have utilized a data-dependent, suspect-screening analytical strategy with HPLC-ESI-HRMS/MS analysis to identify and quantify azobenzene-class disperse dyes in house dust. More than 30 prominent features in house dust were tentatively identified as disperse dyes. Our annotations were assigned using a weight-of-evidence non-targeted analysis approach with multiple open-source, in silico fragmentation algorithms (e.g. Sirius 3.3, MetFrag, MAGMa, CFM-ID, and CSI:FingerID) combined with curated data source, scientific reference, and patent information for each candidate compound. Using authentic standards, we have identified six disperse dyes definitively in our extracts. Prominent compounds identified in dust include the brominated dyes Disperse Blue 373, Disperse Violet 93, and Disperse Orange 61, along with several chlorinated analogs of these structures. Quantitative measurements indicated that these dyes were present in house dust samples at levels up to 10 µg/g. In addition, concentrations of several brominated dyes were highly correlated (correlation coefficient > 0.85) with the flame retardant compound BDE-209 (decabromodiphenylether), although the levels of BDE-209 were typically higher in dust than the quantified disperse dyes. More work is needed to assess the consequences of these measured disperse dye levels in house dust for human exposure, in light of concerns over mutagenicity and allergenicity.

557 Occurrence of Photoinitiators in Human Sera from United States Donors

R. Liu, S. Mabury, University of Toronto

Photoinitiators (PIs) are widely used additives in industrial polymerization processes. Due to their large usage in various products, previous reports showed prevalent occurrence of PIs in sewage sludge and indoor dust. Though humans are likely exposed to PIs via many pathways, including food intake and indoor dust ingestion, no data is available on the occurrence of PIs in humans. In the present study, twenty PIs, including eight benzophenone analogues (BZPs), four thioxanthones (TXs), and eight amine co-initiators (ACIs), were analyzed by UPLC-ESI(+)-MS/MS. Using a newly developed liquid-liquid extraction method, PIs in sera (5 mL) were extracted by MTBE in a 15 mL tube. Recoveries of the PIs in spiked calf serum (500 pg/mL) were 52 – 98 %. Fifty human sera samples were obtained from U.S., which were collected in 2017. Twenty-five samples were from individual male donors, and the others were from individual female donors. Results showed that seventeen PIs were detected, with each serum containing at least seven PIs. The concentrations of ΣPIs were in the range of 423 – 2,870 pg/mL (GM: 836 pg/mL). BZPs were found as the dominant analogues, accounting for 12 – 91 % (mean: 73 %) of ΣPI concentrations, while TXs and ACIs contributed 19 % and 8%, respectively. Significantly higher concentrations of the most frequently detected PIs (DETX, 2-ITX, 4-MBP, EDMAB and PI-907) were found in male sera than female. No relationship was found between age and concentrations of PIs. This is the first reported occurrence of PIs in humans. Further study will focus on the biodegradation of the detected PI analogues.

Community Engagement in Environmental Science – Building Links With Traditional Knowledge and Indigenous Values

558 Are the Fish Safe to Eat? A Collaborative Study of Contaminants in Fish from Kluane Lake, YT

H. Swanson, University of Waterloo / Biology; N. Kassi, Arctic Institute of Community Based Research; N. Zabel, University of Waterloo; B. Branfireun, Western University

Similar to many northern Indigenous groups and communities in Canada, Kluane First Nation, YT, struggles with issues related to food security. The community is located more than 300 km from the nearest grocery store, is currently adapting to changing availability of several subsistence game species, such as moose and sheep. Kluane First Nation approached the Arctic Institute of Community-Based Research to collaborate on development of a food security strategy that would address continuing climatic change, and related changes in subsistence food availability. As part of this strategy, a study was initiated with University researchers to investigate contaminant concentrations in food fishes from nearby Kluane Lake. The lake supports populations of Lake Trout (*Salvelinus namaycush*) and Lake Whitefish (*Coregonus clupeaformis*) that could be used as an alternative food source, but several stakeholders were worried that concentrations of mercury and organic contaminants could be high in these slow-growing northern fishes. In this presentation, we will present results of the contaminant study, but also discuss how the community was engaged, and how issues related to contaminants were communicated to a diverse audience. We will also discuss a highly successful youth exchange, where youth from the community visited University labs to run samples from fish they had captured for mercury analysis. This project functions as an excellent example of community-driven contaminants research and successful two-way knowledge exchange.

559 Increasing Access to Environmental Monitoring Data for Great Lakes Tribes Using Mobile Technology

M. Dellinger, J. Olson, Medical College of Wisconsin; R. Clark, N. Pingatore, Inter-Tribal Council of Michigan; M. Ripley, Chippewa-Ottawa Resource Authority

Background: The presence of Persistent Bioaccumulative Toxics (PBTs) in Great Lakes fish has created a need for fish consumption advisories which can be difficult to interpret, especially if the advice is incongruent cultural fish consumption. The Anishinaabe (Laurentian Great Lakes Ojibwe/Chippewa, Odawa/Ottawa, and Bodewadmik/Potawatomi) are customarily fishing cultures; however, they now consume only one third of the recommended daily fish intake. We partnered with two Inter-Tribal consortia to generate interactive, culturally-tailored fish consumption advice that improves community access to tribal fish contaminant and nutrient data. Methods: A pilot version of the app was generated and given an Anishinaabe name: “Gigiigoo'inaan” [our fish]. The app uses tribal fish harvest data on Mercury (Hg) and Polychlorinated Biphenyls (PCBs) (collected by the Chippewa-Ottawa Resource Authority (CORA)) to generate personalized consumption advice that prioritizes low-PBT, high Omega-3 Fatty Acid (PUFA-3) fish. The software considers bodyweight, age, sex, and selected serving sizes to calculate a ratio of fish grams/meal/bodyweight which can be linked to pre-determined safe consumption ranges using the appropriate guidelines determined by CORA. The partnering organization, Inter Tribal Council of Michigan (ITCM), recruited 24 Anishinaabe adults to pilot the App for usability, influence on dietary behavior, cultural appropriateness, and feasibility of larger implementation. Results: Many (66%) reported that they do not limit consumption of wild caught fish due to contamination although 92% believed they are regularly exposed to contaminants. Almost all, (95%) of participants reported using the internet for food or medical information in the past year, indicating a strong tendency to use new technology for health information. Most (75%) of the participants agreed that the app was useful, culturally appropriate (74%) and helped them identify the best fish to eat (78%). Participants reiterated that consumption advice can be viewed as culturally inappropriate and unwelcome if people feel discouraged from eating fish. Conclusions: An experiment is planned for Summer 2017 to recruit 100 Anishinaabe participants to use Gigiigoo'inaan and select hypothetical fish consumption budgets. We hypothesize, based on this preliminary data, that app users will respond to the culturally-tailored messaging by optimizing their PUFA-3 intake relative to PBTs.

560 Mercury and Arsenic Species in Water, Sediments and Traditional Foods of the Walpole Island First Nation

B. Branfireun, University of Western Ontario / Biology Centre For Environment and Sustainability; N. Williams, Walpole Island First Nation; J. Bend, University of Western Ontario

Walpole Island First Nation, or Bkejwanong, (meaning “where the waters divide”), has had a direct relationship with its surrounding environment throughout time. Surrounded by water, this First Nation Community relies on the St. Clair River for food, water, recreation and for its economic survival. Because of its physical location, down river from Sarnia Ontario's Chemical Valley (a petro-chemical complex in southwestern Ontario, Canada), Walpole Island has been subjected to the effects of both water and airborne pollution for decades. In 1969 industrial mercury contamination was discovered in the St. Clair River sediments which resulted in the closure of commercial fishing activities. This had been the most productive fishery in the Great Lakes and provided full employment to all interested Community members. Persistent contaminants, including the estimated 400 metric tonnes of mercury that were released into the St. Clair River, changed forever the prosperity and well-being of the Community, and remain a major health concern of Community members because the St. Clair River and Lake Erie are sources both of raw and processed drinking water and traditional foods including fish, water fowl and muskrat. The wetlands at Walpole Island are amongst the most extensive and productive in the Great Lakes. Previous community-based research has shown a dramatic decrease in human hair mercury concentrations between 1975 and present (>85% reduction) yet no significant change in blood mercury concentrations, suggesting that there are still ongoing questions related to Community exposure to legacy mercury contamination. Community concerns about continued mercury contamination of sediments, waters and food in traditional fishing and hunting grounds, as well as the effects of arsenic exposure on gestational diabetes, led to the current investigation of mercury and arsenic species in water, sediments and food fishes in the traditional Bkejwanong Territory. This work advances previous studies by using advanced analytical techniques to specifically investigate the speciation of both mercury and arsenic to assess exposure risk. This presentation will report on these findings which are still in progress at the time of this abstract submission, but will also reflect on the collaboration between the Bkejwanong Community and University researchers to address Community health priorities.

561 Risk Evaluation of Contaminated Sediments in Ojibway Territory

M. Buell, Trent University / Environmental and Life Sciences; C.D. Metcalfe, Trent University / Water Quality Centre

Approaches to sediment risk evaluation are yet to include impacted Indigenous communities. Here we evaluate the risk of contaminated sediments by working in partnership with two First Nations Communities in the Bruce Peninsula, Ontario Canada. The aim of our study is to determine the risks of in-place pollutants in Owen Sound Bay, Lake Huron to their traditional fishery of lake whitefish. Traditional ecological knowledge (TEK) and community engagement has been an integral part of how we approached this research; it has informed sampling locations, spawning shoals, and determined our critical receptor species as Lake whitefish (*Coregonus clupeaformis).* We deployed passive sampling devices at these locations and used extracts from these devices to evaluate the risks of these pollutants to developing whitefish embryos as well as a common lab species, Japanese medaka (*Oryzias latipes)* through bioassays. Community participants in this project aided in the sampling process as well as the bioassay development and observations. Our research continues as we develop a sediment risk evaluation process that better encompasses community perspectives by employing TEK to define critical receptors and ecosystem quality. This study demonstrates the effectiveness of community- driven collaborative research projects and their application in ecotoxicological risk assessment.

562 First Nation Community Engagement in Source Water and Land Use Planning

S. Hill, University of Manitoba / Environment

This presentation will share with you some of the experiences of the presenter in working with First Nations communities in Manitoba, Canada over 25+ years of providing research, writing, community engagement and advisory services. It will briefly explain what traditional knowledge entails and the type of issues that need to be considered when doing traditional knowledge research with First Nations communities. While traditional knowledge research is often focused and geared toward a specific issue or development from a governmental or company perspective, there is a wider governance and capacity development consideration when viewed from a First Nation community perspective. Some actual examples of community engagement and traditional knowledge research will be provided for use in source water and land use planning.

563 Community engaged cumulative risk assessment of exposure to inorganic well water contaminants, Crow Reservation, Montana

J.T. Doyle, Little Big Horn College / Crow Water Quality Project; M. Eggers, Montana State University / CBE; M.J. Lefthand, S.L. Young, Crow Environmental Health Steering Committee; C. Martin, Little Big Horn College / Crow Water Quality Project; E. Three Irons, Montana State University / Land Resources and Environmental Science; A. Moore Nall, Montana State University / Earth Science; J. Hoover, University of New Mexico / Health Science Center; J. Bear Below, R.M. Stone, Little Big Horn College / Crow Water Quality Project; A. Camper, Montana State University / Center for Biofilm Engineering

Background: The USGS has shown that exposure to unsafe levels of hazardous metals and nitrate through consuming home well water is a potential health risk for more than 11 million US residents. The federal government does not regulate the quality of home well water; the national scope of the health risk has not been assessed as comprehensive data on home well water treatment and consumption are lacking. On the Crow Reservation in Montana, half of all families drink and/or cook with home well water, despite widespread poor quality, unpleasant taste and community concerns about health risks of consuming the water. Objectives: Tribal members and their academic partners assessed environmental health risks from exposure to well water contaminants by conducting a community-engaged, cumulative risk assessment. Methods: Well water testing, surveys and interviews were used to collect data on well water contaminant concentrations, water treatment methods, well water consumption, and well and septic system protection and maintenance practices. All wells were tested in two seasons, to assess impact of spring runoff on contaminant concentrations. Data were entered into GIS to map and publicize contaminant distributions. All work in Crow was carried out by Tribal members and Crow student interns. Results: Hazard Index calculations show that the water in 23.4% of wells is unsafe due to uranium, manganese, nitrate and/or arsenic. Both natural and anthropogenic sources contribute to contamination. Despite widespread poor taste and odor, 80% of families consume their well water. High levels of secondary contaminants mean that standard water treatment technologies would be very expensive. Most families' financial resources are limited, and less than 5% of participants employ these technologies. Community members identify lack of community health education about well water safety, pre-existing health conditions and limited environmental enforcement as also contributing to their vulnerability. Conclusions: Combining all available knowledge resources through community-engaged research, including Tribal environmental expertise, is essential to researching and addressing Tribal environmental health issues. Ensuring access to safe drinking water is a public environmental health priority for Crow families; this case study is relevant to other rural US families with similarly limited financial and environmental health knowledge resources.

564 Science with Soul

T. Godfery, H. Hireme, Te Whare Wananga O Awanuiarangi / School of Undergraduate Studies

Te Ohu Mo Papatuanuku translates as "a collective response to heal the Earth". This unified approach has been undertaken by tangata whenua (people born of the land) from the Whakatane District of New Zealand, as they attempt to address a legacy of contamination from past timber treatment activities. Enduring collaborative effort has culminated in a project that aims to develop a toolkit for identification and management of contaminated sites, provide knowledge and resources tailored to local needs, and build capacity within the community of science based remediation technology. Whilst both indigenous and scientific groups frequently espouse the value of aligning or integrating Western science and traditional knowledge systems, application of this approach is not always successful. Knowledge systems, whether traditional or contemporary, can result in division and separation – body from mind, heart from soul, human from nature. Conversely, indigenous relationships with environment provide an intrinsic knowing, timeless memory, and sensing wisdom. The role of indigenous being is therefore integral to solving environmental crises and to ongoing environmental care. For Ngati Awa, the indigenous community involved in the Te Ohu Mo Papatuanuku project, relationships with natural phenomena in the form of ancestral connections to our environment, along with genealogical links to each other, inform our behaviour and ensure our actions are bound by an ethos of reciprocity. This presentation examines the journey of Ngati Awa tangata whenua and collaborative partners gathering in response to care for Papatuanuku, remediating environmental contamination whilst also fostering relationships with our environment, and with each other.

565 The Native Environmental Health Research (NEHR) Network: A Model for Service-Oriented Collaborative Research in Tribal Communities

D.K. Stevens, Salish Kootenai College / Life Sciences

There are over 15,000 hazardous waste sites on or next to tribal lands in the US. For tribes with a subsistence culture, there is an increased risk of exposure to toxins while practicing their "tribal lifeways". This has contributed to significant health disparities in Indian Country. Also, due to the extreme under-representation of Native Americans in STEM, tribal governments are often insufficiently staffed with local members or resources to address these issues. That leaves the vast majority of tribes with few Native options. The goal of the Native Environmental Health Research (NEHR) Network is to link tribes with research and human resources at tribal colleges (TCUs) and Native American-serving non-tribal institutions (NASNTIs), in order to help address these pressing environmental health issues. The NEHR Network can mobilize culturally-competent, Native undergraduate and graduate student researchers at participating institutions to help tribal communities, while at the same time, offering these students valuable community-based research experiences that aid with retention in their chosen STEM field. We have been piloting the NEHR model and will present some really positive outcomes. Salish Kootenai College (SKC), a 4-year TCU in western Montana, has been successfully conducting collaborative research projects directly with the Micmac tribe in northern Maine, assessing exposure to mercury from fish and arsenic from private wells. SKC students interact directly with the study community and analyze collected samples back in Montana as part of their degree research. This approach is being further extended to include two UP Michigan tribal communities that also have small, 2-year TCUs. In this case, SKC students and faculty are working with those tribal communities through their 2-year TCU, sharing expertise and building capacity as well as hosting their students for summer internships at SKC. Their own students control the handling of samples and analyses, while SKC students act as near-peer mentors. Finally, NEHR has also funded research at Northern Arizona University (NASNTI) evaluating uranium exposures through consumption of traditional food, such as sheep, on the Navajo Nation. This project is also including students from a Navajo TCU, Dine' College on their project to broaden the collaboration and student impact. This approach is beginning to bear real fruit.

Aquatic and Terrestrial Plants in Ecotoxicology and Risk Assessment

566 Identifying surrogates for listed plant species when data are limited

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More than 20% of the plants in the world are threatened with extinction. In the U.S., this percentage exceeds the proportion of endangered birds and mammals. Causes of listed plant species' declines and the basis for their preservation are incompletely known. This is due primarily to a lack of detailed knowledge regarding plant life histories and corresponding demographics. This widespread lack of data severely limits our ability to model population dynamics under varying conditions in order to inform the decision-making processes concerning threatened and endangered species assessments. Currently, there is a need for better surrogate species to represent listed plants in this regard. Consequently, in this study we use multiple plant databases to develop classification schemes for listed plants for the express purpose of generating surrogates that can facilitate modeling efforts. We explored demographic and eco-physiological databases (COMPADRE and TRY) and linked these to the IUCN Red List for plant species and plant species listed as threatened or endangered under the U.S. Endangered Species Act. Our working hypothesis is that eco-physiological data can inform plant life-history traits when such data are lacking. For example, research demonstrates that "slow" life histories are correlated with large seeds, long-lived leaves and dense wood, while small seeds, short-lived leaves and soft-wood are correlated with "fast" life histories. These associations can be used to estimate traits such as generation time or population growth rate, which are important metrics for extrapolating potential impacts of chemicals (e.g., pesticides) from individual organisms to populations. Here, we use matrix population models to generate demographic parameters and elasticity analyses for all the species compiled. Statistical tests were developed to make comparisons between listed and non-listed species and to search for classification patterns among listed species. Our approach, which can be expanded to other taxonomic groups, provides insight into the biological basis for differences in species vulnerability to environmental stressors.

567 Risk assessment of Amaranthus spinosus samples contaminated with pesticides residues in Wukari-Donga axis Taraba State, Nigeria

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There is a significant economic diversification policy shift from crude oil dominated economy to agro-based one in Nigeria, leading to increased use of pesticides and obsolete chemicals in agriculture. This practice has resulted in pollution of the environment as well as contamination of agricultural products. Exposure to pesticide residues may pose a lot of challenges to the health of consumers of such agro products. Thus, in this study, we analyzed selected pesticide residues in fresh leafy vegetables (*Amaranthus spinosus*) harvested from Donga, Taraba State. High performance liquid chromatography (HPLC) was used to determine the concentration of selected pesticide residues in the harvested leafy vegetables. The results showed that nineteen pesticide residues; cyclodienes, cyclohexanes, DDT, DDD, dicofol, perthane, endosulfan, pentachlorobenzene, mirex, toxaphene, hexachlorobenzene, methoxychlor, aldrin, dieldrin, alpha-HCH, beta-HCH, gamma-HCH, chlordane and heptachlor were present in the analyzed samples in the range of 0.360 - 1.866 µg/kg. Pentachlorobenzene had the lowest mean concentration (0.360 ± 0.063µg/kg) and gamma- HCH having the highest mean concentration (1.866 ± 0.483µg/kg). The order of pesticide residue concentrations in the analyzed samples was gamma-HCH ? alpha-HCH ? methoxychlor ? beta-HCH ? DDT ? DDD ? perthane ? cyclohexane ? toxaphene ? mirex ? cyclodienes ? chlordane ? aldrin ? heptachlor ? dieldrin ? endosulfan ? dicofol > hexachlorobenzene > pentachlorobenzene. Generally, the mean concentrations of all the pesticide residues detected in the analyzed samples were above the maximum residue limits set by FAO and WHO. The mean concentrations of DDT and its metabolite, DDD in the analyzed samples were higher than the WHO and FAO MRLs of 1.0µg/kg. Also, the mean concentrations of Dieldrin and Aldrin were found to be higher than the WHO/FAO MRLs of 0.2µg/kg while the mean concentrations of alpha-HCH, beta-HCH, and gamma HCH, were also observed to exceed the MRLs of 0.01µg/kg set by WHO/FAO. Therefore, the presence of some banned pesticides like DDT may pose greater health challenges to susceptible populations among children and pregnant mothers.

568 Population model for the Mead's milkweed: A tool for pesticide risk assessment for a threatened plant

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Population models can address the potential impacts of pesticides on populations or species rather than individuals, and have been identified as necessary tools for pesticide risk assessment of species listed under the Endangered Species Act (ESA). Few examples of population models developed for this specific purpose are found in the scientific literature, especially population models addressing potential risks of pesticides to listed plants. We present a population model for Mead's milkweed (*Asclepias meadii*), a species listed as threatened under the ESA throughout its range across the Midwestern US, as an example of a long-lived and slow-reproducing herbaceous plant species. With the model, we test different herbicide dose-response curves as derived from standard test species to assess a range of realistic organism-level responses and their relationships to population-level outcomes. We combine assumptions about organism-level toxicity of the herbicides with realistic exposure scenarios over extended time periods. Population dynamics and abundances over time with and without exposure to herbicides are compared. With the population model of the listed milkweed, we can estimate potential effects of herbicides to populations which represent an ecologically relevant endpoint for risk assessments. Scenarios relating to the toxicity of pesticides to the species, spatial and temporal exposure patterns, and assumptions about other stressors affecting populations of the species can be assessed. To assess hypothetical mitigation scenarios, buffers (i.e. setback herbicide spraying distances from species locations) are imposed within the model in order to evaluate their corresponding influence on population metrics as a function of distance.

569 The Concept of Recovery in Plants from Exposure to Chemicals

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The plant interest group of SETAC has a committee working on the topic of recovery and this presentation represent current thought on the topic. When we evaluate the risk of chemicals to plant species or communities we determine both lethal and nonlethal effects. However, when we observe effects we do not consider whether the effects are transient or persistent. The ability of plants to recover after the exposure to a chemical is important to consider when evaluating effects on populations and/or conducting a risk assessment. For example, a young plant in a vegetative vigor study may show leaf damage within a day after it is sprayed, but after two weeks of growth that damage may no longer be apparent as old damaged leaves have senesced and only new unaffected leaves are visible. In algae or lemna studies, an aliquot of cells can be transferred to untreated media at the end of a test and after several days, the growth rates of the affected groups may approach that of the controls indicating recovery. In more complex mesocosm studies the concept of recovery is even further complicated by seasonality, changes in nutrients, recolonization, competition, and other factors. Terminology and methodology need to be standardized if the concept of recovery is incorporated into evaluations of chemicals. For some plant types and properties, recovery is contingent upon the timing and duration of exposure and extent of injury. In such cases, the definition of recovery must specify timing and duration of exposure in the operational definition. Examples of recovery in laboratory studies for algae, lemna, and myriophylum studies will be presented as well as results from mesocosm studies. Statistical procedures and experimental designs will be presented for these examples and interpretation of results will be discussed.

570 Organic chemical uptake in terrestrial plants: Experimental variability

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Experiential data describing organic chemical uptake by plants are often expressed as ratios of chemical concentrations in the plant compartments of interest (e.g. leaves, shoots, roots, xylem sap) to that in the exposure medium (soil, soil pore water, hydroponic solution, air). These ratios are often referred to as bioconcentration factors (BCFs) but have also been named for the specific plant compartment sampled such as root concentration factors (RCFs), leaf concentration factors (LCFs), or transpiration stream (xylem sap) concentrations factors (TSCFs). All plant uptake data are dependent on the experimental measurement approaches and the fate processes within plants and exposure media. We collected and reviewed measured data from over 350 papers that included 310 organic chemicals and 112 plant species. Since there is little regulatory guidance, plant uptake studies were conducted using a variety of experimental approaches that make inter-study comparisons difficult. Key parameters describing exposure and plant growth conditions were often missing and units associated with the plant BCFs were not always reported or were unclear. The lack of comparable data also limits model evaluation and development. Standard test protocols or standard reporting guidelines, for the measurement of plant uptake data are recommended to generate comparable, high quality data that will improve mechanistic understanding of organic chemical uptake by plants. However, despite data comparability issues, there were some general trends that could be discerned and it was found some widely used log K_{ow} based models tended to significantly under predict plant uptake.

571 Acetaminophen detoxification in plants via induction of glutathione S-transferases

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Soil contamination with pharmaceuticals is an emerging environmental concern, especially on agricultural lands that receive treated wastewater, biosolids and animal wastes. Research on the impact of pharmaceuticals in the environment has mainly focused on determining persistence, transport and concentrations in the soil; little work has been conducted to elucidate the mechanisms of pharmaceutical uptake, accumulation and detoxification by plants. Here, a hydroponic experiment was conducted to investigate the uptake, translocation and metabolism of acetaminophen in cucumber over 144 hours. Acetaminophen was taken up by cucumber and underwent rapid metabolism. After 12 h of exposure, glutathione-conjugated metabolites in cucumber tissues were identified, but were not detected in xylem solutions. Significant increase in the activities of glutathione S-transferases (GST) and peroxidase (POD) were observed during the exposure, suggesting their roles in acetaminophen metabolism in cucumber. The concentrations of acetaminophen, glutathione-conjugated metabolites as well as activities of GST and POD were highest in roots, suggesting an intensive transformation of acetaminophen in roots. Glutathione depletion can become a problem when detoxifying large quantities of xenobiotics. Here, glutathione rapidly decreased and then gradually recovered. The activities of γ-glutamylcysteine synthetase and glutathione reductase increased significantly after acetaminophen exposure, indicating that a rapid recycling or synthesis of GSH may be responsible for keeping the glutathione pools. The detoxification mechanism by GST was also detected in tomato, wheat, bean and afalfa, and vegetable grown in biosolids as well as at real environment low concentrations of acetaminophen. In summary, our study reveals that the ability of GST to detoxify pharmaceuticals is a common route for in plants.

572 Digging Deeper: Selenium uptake in plants and protein regulation determine plant suitability for phytoremediation

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Selenium (Se) has emerged as a contaminant in terrestrial and aquatic environments in many regions around the world as a result of anthropogenic activities such as mining. Selenium is a naturally occurring element and within a narrow range is essential for healthy human, animal and microorganism functioning. To date, limited research has been conducted to determine how the patterns of uptake and accumulation determine the suitability of plants for phytoremediation of mine sites. The plants, *Brassica rapa* ‘Vitamin Greens’, *Helianthus annus* ‘Dwarf Sunflower’ (secondary selenium accumulators) and *Neptunia Amplexicaulis* ‘Selenium Weed’ (a selenium hyperaccumulator), were grown in tissue culture and harvested after 21 days of exposure to a range of increasing concentrations of selenite and selenate salts of 5-50 µM and 10-100 µM respectively. Sub-lethal exposure concentrations were selected for further studies. After a series of comparative enzyme and metabolite assays were completed, Se^{75} autoradiography and cell fractionation studies were conducted on plant leaves, stems and roots and analyses of plant samples for differential display of the proteins up/down regulated were performed. It was found that the selenium hyperaccumulator is unlikely to incorporate selenium-substituted amino acids but instead, accumulate selenium in the form of non-protein amino acids while the secondary accumulators contain misincorporated proteins.

573 Exploring the roles of iron and carbon in controlling sulfide toxicity in multi-year wild rice mesocosms

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In anoxic sediment of a wild rice bed, sulfide is produced when sulfate is reduced by sulfate reducing bacteria in the presence of organic carbon but may then precipitate with dissolved, ferrous iron to form non-reactive iron sulfide precipitate. To investigate the interactions of sulfate, iron, and carbon loading, sulfate and iron were added to and litter was removed from wild rice mesocosms in a factorial design over four growing seasons. Porewater iron and sulfide concentrations were measured with rhizons twice yearly. Solid phase iron and acid volatile sulfide (AVS) concentrations were measured in the sediment after the second growing season, and on roots at the end of the third growing season. Sulfate appears to be the controlling factor in sediment geochemistry, as it was the only factor that significantly affected AVS and iron on sediment and roots. Sulfate addition decreased the number of emerged seedlings that survived early in the growing season by 80% and decreased the number and weight of seeds produced at the end of the growing season. Litter and iron alone did not affect wild rice seedling survival or seed production, but sulfate addition and iron addition together decreased the number of emerged seedlings by only 50%. Interestingly, iron addition did not significantly affect bulk sediment iron and sulfur geochemistry, but partly ameliorated the effects of sulfide on wild rice seedlings, suggesting that the mechanism by which iron mitigates sulfide toxicity may be confined to near the rooting zone and may only occur early in the life cycle of wild rice.

More Data Is Not Always Better – Using Weight-of-Evidence Approaches in Environmental Risk Characterization

574 A Framework for Inference by Weight-of-Evidence in Ecological Assessments

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The U.S. Environmental Protection Agency's Risk Assessment Forum has developed a framework and guidelines for inference by weight of evidence. Like the Agency's Guidelines for Ecological Risk Assessment, these guidelines are sufficiently general to be adapted to a wide range of uses while having sufficient structure and guidance to create common standards of practice. Potential uses include determining whether a chemical has a particular property (e.g., bioaccumulative), what hazard a chemical poses, or what agent caused an observed impairment. The framework has three basic steps: assemble evidence, weight the evidence, and weigh the body of evidence. The assembling evidence step includes systematic literature reviews, generation of new information, screening, categorization, and data analysis. The weighting of evidence step is based on three properties: relevance, strength and reliability. The framework employs a scoring system that makes weighting formal and explicit without pseudo-quantification of the qualitative properties. The weighing the body of evidence step involves integrating the evidence, weighing the body of evidence, and interpreting the evidence, including any ambiguities and discrepancies. This framework is used to infer qualities such as impairment, but it can be extended to derive quantities such as criteria or levels of effect. The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the USEPA.

576 Metals Bioaccumulation in Marine Environments: Using a Weight-of-Evidence Approach to Delineate Source Media for Risk Management Decision-Making

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Correlating site contamination in a given medium to tissue concentrations can be more complex than finding relationships between chemistry and direct toxicity in sediment benthos. A number of variables must be considered when assessing the bioaccumulation pathway, including chemical-physical properties (e.g., solubility, partition coefficients), biological considerations (e.g., species, habitats), sediment and water characteristics, and tidal action. Using a weight of evidence (WOE) approach to address relative source contributions of metals in site tissue can result in focused investigations and risk assessments, and cost savings. A case study will be used to demonstrate an approach designed to collect meaningful data from a marine habitat to assess the source inputs causing the bioaccumulation of cadmium in clam tissue as a result of contaminant releases at a former plating shop. Specifically, the difficult question about whether marine sediment or groundwater discharging as seeps to the intertidal environment is primarily responsible for cadmium levels in clam tissue was addressed. The project site consists of a man-made, self-armored cobbly beach that is inundated at high tide, with seeps visible at low tide. Long-term monitoring of seeps, sediment and clam tissue has occurred since the 1994 record of decision (ROD). The goal of the post-ROD risk assessment was to meet the ROD objective by evaluating the need for control measures to address metals contamination. Data collection focused on evaluation of media most likely contributing to metals bioaccumulation in clams to address the shellfish consumption pathway. Thus, a WOE approach included the following: acid-volatile sulfide/simultaneous extractable metals testing, utilizing existing biological survey information, targeting an appropriate shellfish indicator species, understanding the selected species life history, and planning data to address other contaminant sources to the beach (e.g., outfall discharges). As a final point of interest, this presentation will include a comparison of the site-specific tissue data to modeled tissue concentrations based on literature-derived bioaccumulation factors for water and sediment. We will also discuss the conclusions that could have been drawn if only surface water, seep and sediment data had been used to model tissue levels to assess the relative importance of seep water versus sediment cadmium levels.

577 Implementing a weight-of-evidence approach for sediment quality management in California: Lessons learned from regional monitoring and TMDLs

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A regulatory sediment quality assessment framework based on weight of evidence (sediment quality triad) has been implemented in California since 2009. Application of the framework to 15 years of regional monitoring data (1998-2013) in southern California coastal waters provides a unique data set to examine both the power and limitations of weight of evidence analyses for understanding regional and temporal changes in sediment quality and the influence of multiple environmental stressors. Chemistry, benthic macrofauna, and toxicity analyses of 346 sediment samples from the southern California shelf, submarine canyons, bays, and estuaries in 2013 indicate that sediment quality is relatively good. Indices used for assessing attainment of the Water Board's sediment quality objective for protection of aquatic life show that approximately 98% of the southern California Bight area has no or marginal impacts from contamination, although sediments in bays and estuaries still show evidence of greater contamination and toxicity than is observed in offshore areas, an indication of the influence of contaminant inputs from multiple current and legacy sources, including watershed runoff, industrial discharge, and commercial and recreational activities. Integrated assessment of sediment condition was essential to identifying substantial improvement in overall sediment quality since 1998, indicating that management actions to improve water quality have been effective in the region. However, temporal trends in each line of evidence showed different patterns, with evidence of improvement in toxicity, little change in chemistry, and a slight decline in benthic community condition. There was a relatively low association between biological effects and specific contaminants, indicating that many important stressors are not being evaluated or that current measurement methods are poor indicators of exposure to biota. Application of weight of evidence approaches in TMDLs presents substantial challenges, as guidance for developing chemically-based cleanup targets from an integrated assessment is rarely available. Recent progress in addressing those challenges will be discussed.

578 Integrating Weight-of-Evidence Into Toxicity Benchmark Development: Trichloroethylene Example

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Developing toxicology benchmarks for applications in risk assessment is rarely straightforward. Often, interpreting an abundance of toxicology data can be more daunting than dealing with a limited data set. Differences in study design, animal models, methods of administration, variability in response and statistical error all contribute to variations in interpretation. Current methods often assume equivalent quality between studies and available data and use the most sensitive outcome to be protective of others. Here we present a method to score studies based on scientific rigor, applicability, and study quality to help ascertain false positive and false negative results in an abundance of data for trichloroethylene (TCE). Each study that was considered sufficient to develop a point of departure was scored between 95-50 where 13 studies were considered high confidence, 7 studies were of medium confidence, 13 of low confidence, and 5 were in the “do not use category”. This approach is a first step in allowing developers to assess the relative value of negative data in an objective manner and begin to assess the influences of false negative and false positive relationships.

579 Integrating Toxicological Data with BISCT and GRAVEE for Better Chemical Assessments

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As the amount of information about chemicals continues to grow, risk assessors are challenged with integrating this information. This has become more challenging as the number of high throughput screening assays grows and as toxicologists use more high content approaches such as toxicogenomics. Making accurate predictions for screening, or assessing potential toxicity, has not become any easier using traditional approaches. BISCT was developed to create probabilistic likelihoods of an adverse outcome occurring based on a causal network of molecular and systems-level events. BISCT uses causal adverse outcome pathway networks (CAOPNs), and user-supplied assay, biomarker, or other data, and generates a probability of adversity. The advantage of BISCT is that because it is a Bayesian Network, it already performs the weight of evidence functions due to the nature of the causal network itself (i.e., the probabilities between each of the nodes represents the weight of evidence on the way to the adverse outcome). If we have exposure-response data for key events that are sufficient to drive the adverse outcome (either based on biological knowledge or causal network calculus), then we can generate a probabilistic point of departure (POD) using Monte Carlo simulations, and use a single-compartment reverse toxicokinetic model (parameterized using quantitative structure activity relationship models if necessary) and reasonable uncertainty factors to make our GRAVEE (good risk assessment values for environmental exposures). We will demonstrate this approach using our CAOPNs for DNA damage, oxidative stress and fish fecundity disruption for the chemicals benzo[k]fluoranthene, TNT, and several estrogenic endocrine disruptors.

580 Use of a Population-Based Approach to Assess the Influence of Genetic Variation in a TCDD-induced Dose-Response Relationship

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Traditional toxicology studies do not typically account for interindividual variation within the human population. As chemical exposures occur within heterogeneous populations, there is a disconnect between the results provided by common laboratory models and the data required to assess risk posed to the human population. Recently, the National Research Council (NRC) has hypothesized that, when accounting for interindividual variability within the human population, the low-dose region of dose-response relationships (DRRs) would effectively linearize for non-cancer endpoints. Given the implications of a linear model (i.e. no threshold) within risk-assessment and the lack of data to support the NRC’s hypothesis, this project focused on addressing the role of genetic variability in influencing the shape of the low-dose region of a 2,3,7,8-tetrachlordibenzo-*p*-dioxin (TCDD)-induced DRR. First, B cells were isolated from 52 human donors, activated with CD40-ligand, and exposed to various concentrations of TCDD. In averaging all donors, results indicate a significant decrease in the number of cells secreting Immunoglobulin-M (IgM; $p < 0.05$). In modeling the low-dose region, nonlinear statistical models best fit the empirical results suggesting that the NRC’s hypothesis may not fit all DRRs. More interestingly, we found profound interindividual variability in the population of donors with differences of up to 73 fold at the 30 nM TCDD. Second, to investigate potential genetic modifiers that drive such interindividual variability, we isolated B cells from a panel of 14 genetically-diverse mouse strains ($n > 3$ per strain) and exposed to TCDD as done with the human B cells. Results indicated that, while all strains had decreased numbers of B cells secreting IgM, 4 had significant decreases ($p < 0.05$) suggesting the presence of interstrain variation. In scanning for quantitative trait loci, we identified a region on chromosome 1 that is associated with the TCDD-induced inhibition of the number of cells secreting IgM. Preliminary results along with previous published reports suggest that several candidate genes within this region have potential to alter TCDD-induced immunotoxicity and appear correlated with TCDD-mediated gene-expression in murine and human B cells. Thus, using population-based models, we were able to model the low-dose region of a TCDD-induced DRR and identify several candidate genes that have potential to affect individual’s susceptibility to TCDD.

581 Practical Application of Weight-of-Evidence in Human Health Risk Assessment of Arsenic Contaminated Mine Tailings

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This presentation is an example of the practical application of lines of evidence to support the conclusion that arsenic contamination in mine tailings at a site in Oregon have a relative bioavailability of less than 10 percent. Use of these lines of evidence resulted in a significant reduction in the scope of remedial action required, as well as significant cost savings. The example is an envrionmental cleanup project in Oregon referred to as the Red Rock Road. The site is a 17-mile long former railbed used historically for timber operations. No longer in use, the railbed is now located on approximately 140 individual properties of varied land uses, including residential and occupational. Studies that were conducted to support the conclusions with regard to reduced arsenic bioavailability in site soils included selective sequential extraction (SSE) of the soil, the Relative Bioavailability Leaching Procedure (RBALP), which is an in vitro extraction test used to estimate relative oral bioavailability of chemicals from soil, and speciation and mineralogy by electron microprobe analysis (EMPA). The results for soils from this site were compared with data from numerous soils from other sites that had been studied for bioavailability using animal studies. Together, these studies supported the conclusion that soils along the 17 miles of road in the Site showed low (< 10%) relative bioavailability of arsenic. This information was used to refine the human health risk assessment for the site, which then indicated that most areas within the site did not require remedial action in order to meet goals for human health protection. This presentation will discuss the lines of evidence that supported the conclusions with regard to arsenic bioavailability from site soils, and the impacts on the scope of remediation required for the site.

The Emerging Concept of Neuroendocrine Disruption – More Than Hormones Are Upset

582 Environmental Estrogen Exposure Is Correlated with the Size of a Sexually Dimorphic Brain Region in the Painted Turtle (Chrysemys picta)

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Shallow lakes are distributed throughout Minnesota, and are impacted by a variety of land uses. Some of these uses contribute estrogen-mimicking compounds to the lakes, which may impact the health of wildlife populations. To explore whether differences in environmental estrogen exposure might impact wildlife in Minnesota's shallow lakes, we examined vitellogenin (VTG) levels in the painted turtle (*Chrysemys picta*) as a marker for environmental estrogen exposure, and measured the size of the nucleus paraventricularis (NPV) in the brain, which has been shown to be sexually dimorphic in turtles (larger in males than females). We collected blood and/or brain samples from 136 turtles, obtained as by-catch from gill nets stretched across 71 shallow lakes in Minnesota. We developed a novel, species-specific antibody-capture competitive ELISA for painted turtle VTG. Sections of brains (40 µm) were stained with cresyl violet, dehydrated, and coverslipped, and the NPV was traced by experimenters blind to the sex and lake origin of the turtles. Our results show that average turtle VTG concentrations varied widely across lakes in Minnesota, from less than 1000 µg/mL to over 6500 µg/mL. Overall, VTG levels were significantly higher in female turtles than in males (student's *t*-test $p < 0.025$). For NPV size, we compared turtles obtained from lakes with the lowest VTG levels (low-VTG lakes) with those from lakes with the highest VTG levels (high-VTG lakes). In low-VTG lakes, the average size of the NPV in males was significantly larger than in females ($p < 0.01$), as previously reported. However, in high-VTG lakes, the average NPV was significantly smaller in males than in females ($p < 0.025$). Moreover, VTG levels and male NPV size showed a strong negative correlation within turtles collected from these lakes ($R^2 = 0.51$; $p < 0.002$). Our results suggest that environmental estrogen exposure impacts brain structure in painted turtles in a sex-dependent manner. We are currently investigating the extent to which such exposure is related to watershed land use, whether NPV size changes in response to environmental estrogens, and whether changes in NPV size have behavioral or survival consequences for the painted turtle. Funding provided by the Minnesota Environment and Natural Resources Trust Fund, as recommended by the Legislative-Citizen Commission on Minnesota Resources.

583 Calling behavior as non-invasive endpoint for the detection of endocrine disrupting chemicals? XENOCALL as an example

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During the last decades evidence has accumulated demonstrating that anthropogenic chemicals, which can interfere with the endocrine system of vertebrates, can affect a wide range of behaviors, such as mating and other reproductive behaviors, but also activity and motivation, communication, and other social behaviors, such as aggression and dominance. These so called endocrine disrupting chemicals (EDC) primarily accumulate in surface waters. Hence, fishes and amphibians are the main (aquatic) vertebrates being affected by the diverse adverse effects of those substances. In the frame of regulatory risk assessment, selected species (mostly fish) have been used in in vivo experiments for the assessment of environmental hazards, mainly focusing upon apical endpoints, such as death, growth and reproduction. Those existing biomarker tests, however, imply the sacrifice of the experimental animals; valid non-invasive testing methods do not exist, yet. Consequentially, we recently examined whether exposure to EDC with (anti)androgenic and (anti)estrogenic modes of action can affect the calling behavior of male African clawed frogs (*Xenopus laevis*). The obtained results indicate that the calling behavior can be used as valid biomarker for the detection of (anti)androgenic and (anti)estrogenic EDC, as this behavior is modulated distinctively when animals are exposed to these chemicals, even at environmentally relevant concentrations. Exposure to non-EDC, on the other hand, did not lead to those specific effects. Thus, just by looking for characteristic changes in the calling behavior of the frogs, which are potentially due to neuroendocrine disruption of vocal pathways in the CNS, (anti)androgenic and (anti)estrogenic EDC can be detected and assessed non-invasively but accurately.

584 Impacts of thyroid disruption on the reproductive behavior of fathead minnows

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Previous studies in our lab have shown that early-life-stage exposure of fathead minnows (*Pimephales promelas*) to the model thyroid disruptor propylthiouracil (PTU) lead to a significant decrease in total fecundity during a 21-day breeding assay conducted after maturation. This decrease was associated with a significant decrease in spawning frequency indicating that the reproductive behavior of exposed fish may have been disrupted. However, there is limited available information on how early-life-stage thyroid disruption affects behavior in the fathead minnow. Given that thyroid disruptors are prevalent in the environment and the reduction in fecundity of PTU-exposed fish during the breeding assay, there is a need to investigate the potential for thyroid disrupting compounds to alter behavior. As such, this experiment was conducted to gather further information on the potential behavioral effects of early-life-stage exposure to PTU. Minnows were exposed to PTU during early development and the developmental expression profiles were monitored for genes related to brain sexual differentiation and behavior in control and exposed fish. Expression of several genes was found to be altered in brains of PTU-exposed fish. For example, aromatase, known to affect reproductive behavior in birds and mammals, and several other genes related to sex steroid signaling were downregulated in PTU-exposed fish at the end of the exposure. Expression of basic transcription element-binding protein, a transcription factor involved in neurodevelopment, was also reduced in exposed fish. This data, in conjunction with the results from the previous breeding assay and trials of male reproductive behavior, demonstrate the potential for developmental exposures to thyroid disrupting compounds to alter neuroendocrine development and behavior.

585 Alterations of organizational and activational effects of environmental contaminants in fish

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Organizational programming of the brain in vertebrates occurs early in development by the action of steroid hormones, which masculinize or feminize the brain. This establishes the neural circuity that can later be activated by surges in steroid hormones during puberty and adulthood. However in fish, the brain demonstrates greater plasticity than in other vertebrates as in adults, the brain can undergo reprogramming to change from being a masculinized to a feminized profile and vice versa. These waves of programming and reprogramming events are modulated by the addition of exogenous steroid hormones, thus bringing into question whether fish exhibit the same organizational/activational programming observed in mammals. This is further complicated by the observation that some fish lack genetic sex determination and rely instead on temperature and other environmental cues for sex determination. High levels of neurogenesis in fish brains through adulthood also contribute to the extreme plasticity observed. Exposure of fish to neuroactive contaminants in the environment thus affects brain plasticity with profound effects on their behavior. Contaminants in the environment that likely alter brain programming in fish include estrogens, androgens, gestagens, glucocorticoids and neuroactive pharmaceuticals, such as prozac. The fundamental pathways that are involved in organizational/activational changes in the brain of fish and that can extend throughout the lifespan of some fish species are not known. Gene and protein expression profiling of response to hormones and contaminant exposures is beginning to elucidate biochemical and signaling pathways that may be involved. By disrupting these pathways and brain sexual differentiation in fish, environmental contaminants might compromise key sexual behaviors, thereby reducing the likelihood that exposed fish may reproduce, which could ultimately result in population declines.

586 Neuroendocrine Disruption of Organizational-Activational Brain Programming in Reptiles

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Brain sexual differentiation is orchestrated by steroid hormones during embryonic development and in some taxa this can extend into adulthood. This concept, which has been referred to as organization-activational programming, can lead to adult behaviors that occur in a sex-specific manner. Neuroendocrine disruptors (NEDs) may act to affect endogenous neurohormones and neuropeptides and/or their receptors, which may result in males demonstrating female-typical behaviors and vice versa. Although there are reports on NED exposure in fish, less information is available on the direct effects of NEDs on reptilian brain development. Certain reptilian species lack sex chromosomes and thus depend upon temperature sex determination (TSD) rather than genetic sex determination (GSD). While brain programming in certain fish species demonstrates significant plasticity with some males reverting to females in later life and vice versa in other piscine species, it is not clear whether organizational-activational effects of steroid hormones in reptiles induces permanent brain programming. Several reports have shown that developmental exposure to endocrine disrupting chemicals (EDCs) can affect gonadal sexual differentiation and subsequent production of steroid and other gonadal hormones that in turn can affect brain sexual differentiation. Disturbances in these physiological responses could be potential mechanisms by which NEDs alter neurobehavioral programming in reptiles. Recently, it has been shown that developmental exposure of painted turtles (*Chrysemys picta*), who demonstrate TSD, to the EDCs, bisphenol A (BPA) and ethinylestradiol (EE2) can likely induce permanent neurobehavioral effects. Individuals incubated at the male-permissive temperature and treated with either BPA (1 ng/μl) or EE2 (4 ng/μl) demonstrated improved spatial navigational abilities compared to vehicle control group. Gene expression analysis of the brains from the same turtles revealed that *in ovo* exposure of turtles to BPA increases transcripts associated with ribosomal and mitochondrial functions, especially bioenergetics, and these pathways correlated with improved behavioral performance. The NED-induced brain reprogramming may result in individuals - who should otherwise be male - failing to exhibit male-typical courtship behaviors, which could reduce the likelihood of reproducing and ultimately lead to population declines and ecosystem level effects.

587 Endocrine Disrupting Chemicals and Neural Progenitors: Do EDCs Mimic Hormones or Act in Unexpected Ways?

D. Nesan, H. Thornton, C. Kinch, University of Calgary; H.R. Habibi, University of Calgary / Biological Sciences; D. Kurrasch, University of Calgary

Previously, we showed that zebrafish exposed to the plasticizer bisphenol A (BPA) during early brain development demonstrated precocious neurogenesis and a shift in the timing of neuronal birth specifically within the hypothalamus. Given that BPA is thought to act via sex steroid receptors, these results suggest that hormone signaling might play an unappreciated role in deciding progenitor behavior. Thus, we decided to take a step back and study the endogenous role of hormones themselves in neural progenitors, as an entry point to assessing the adverse effects of EDCs. To do so, we have created an in vitro neurosphere assay whereby hypothalamic neural stem cells are isolated from E12.5 mouse brains and grown in culture. We can treat these neurospheres with exogenous sex steroids (estradiol and testosterone, primarily) as well as EDCs, and study their effects on proliferation and differentiation. Our preliminary data shows that these hormones indeed influence hypothalamic progenitors and is consistent with our hypothesis that EDCs can interfere with normal brain development by disrupting hormone signaling in these cells. Our current work is focused on understanding how hormones change progenitor proliferative potential, and to determine whether EDCs mimic these same sex steroid signaling pathways or act via unexpected mechanisms.

588 Transgenerational disruption of the stress response in zebrafish from developmental exposure to fluoxetine

M. Vera Chang, T.W. Moon, V. Trudeau, University of Ottawa / Biology

Neuroactive pharmaceuticals confer major therapeutic benefits for disorders such as anxiety and depression. The detection of a wide range of pharmaceuticals in the aquatic environment has made it evident that these chemicals are constantly released to the environment, and may pose risks to non-target organisms. In the last decade, our work on the selective serotonin reuptake inhibitor (SSRI) fluoxetine (Prozac) has provided strong support for the neuroendocrine disruption hypothesis. Fluoxetine causes multiple levels of negative effects in teleost fish — from reproductive problems to reduced feeding and metabolic upsets. We now report that early life exposure to fluoxetine decreases the endocrine stress response (whole body cortisol) in zebrafish, an effect that persists across at least 4 generations. Embryos/larvae of a clean population were exposed to fluoxetine (0.54 and 54 micrograms/L) for the first 6 days of life during the formation of the serotoninergic system and then descendants were raised in clean water under constant rearing conditions for 4 generations. For the duration of the study, we observed a persistent blunted cortisol response in the exposed-males subjected to the net stressor ($p< 0.005$). Upsets in cortisol levels can be associated with behavioural alterations, so we quantified diverse exploratory behavioural parameters using the novel tank paradigm. Males of F0-exposed fish, and F1-F2 progenitors displayed reduced exploratory behaviour ($p< 0.05$). This reduction is likely associated with the decrease in cortisol levels because chemical-induced reduction of steroidogenesis with the inhibitor metyrapone also similarly reduced the exploratory behavior of the fish. As we enter a period of increasing usage of antidepressants, these data are a cause for concern for the potential long term negative impacts on neuroendocrine systems in aquatic organisms or humans exposed to these therapeutic drugs. Acknowledgements: FRQNT scholarship (MVC) and grants from NSERC (TWM, VLT), uOttawa (TWM), and University Research.

Ecosystem Services, Stakeholder Values and Sustainability

589 Global Ecosystem Service Assessment

K. Brauman, University of Minnesota / Institute on the Environment

Assessment of ecosystem services, the benefits nature provides to people, is usually local, undertaken the context of land use planning, regulatory assessment, and strategic deployment of resources. But demand is growing for large-scale assessments. Ecosystem accounting takes place at the national scale. Multi-national companies are worried about their impacts and interested in strategic siting. Inter-governmental agreements from the Sustainability Development Goals (SGD) to commitments to reducing deforestation and degradation (REDD++) for climate stabilization rely on global assessment. The Intergovernmental Platform on Biodiversity and Ecosystem Services (IPBES) is mid-way through a global assessment. Unlike carbon, however, the benefits of many ecosystem services, such as water-related services, are local to regional, not global, and ecosystem effects vary geographically. Yet demand for global information persists. In addition to an overview of current global ecosystems service efforts, I will discuss ongoing work to evaluate and map ecosystem services, with a focus on hydrologic ecosystem services. A process to distinguish criteria that identify places where hydrologic services are likely to be valuable is laid out. These criteria are both biophysical and social, seeking to identify regions where ecosystem services play a large role in regulating water, where there is demand for that impact on water, and where a change in service delivery is likely. Maps of these characteristics indicate regions likely to benefit from management of hydrologic ecosystem services.

590 Assessment and management of risks to ecosystem services: European status

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The benefits which flow from natural capital, termed ecosystem services (ES) are under threat from habitat loss, climate change, invasive species, over exploitation and pollution. Consequently they are increasingly the focus of environmental policy. A stated objective of the EU 7th Environment Action Programme is 'to protect, conserve and enhance Europe's natural capital' and the 2020 Biodiversity Strategy has a headline target of 'halting the loss of biodiversity and the degradation of ecosystem services in the EU by 2020'. However, questions remain: do we have sufficient knowledge and understanding to assess, predict and manage the effects of anthropogenic activities on ES delivery in multifunctional landscapes that are often exposed to multiple pressures, and are the monitoring, regulatory and decision frameworks that have developed over decades fit for purpose as we seek to apply this evolving knowledge? Assessing and managing the risks that stressors, often introduced as people seek to obtain selected services from land- and water-scapes, pose to the sustainable delivery of these and other ES is a transdisciplinary challenge requiring the development of new approaches to cross-disciplinary communication and collaboration. A recent SETAC Europe special session explored how close we are to adopting an ES approach to assessing and managing environmental risk from anthropogenic stressors. It drew on the findings of a series of SETAC-Europe sponsored workshops (Chemicals: Assessment of Risks to Ecosystem Services) and built on the recent work by EFSA and ECETOC in using ES approaches to develop specific protection goals and the outcomes of the joint ESA-SETAC Pellston workshop Ecosystem Services, Environmental Stressors and Decision Making. Thus, it reviewed the state of the practice for the use of ES (primarily) in Europe, for prospective risk assessment, chemicals regulation, retrospective risk assessment (also termed forensic ecology), remediation and restoration. This paper will summarize key points, focusing on progress, opportunities, gaps and challenges.

591 Ecosystem Services in the Great Lakes - Results of a Summit

W.R. Munns, USEPA / Atlantic Ecology Division; B.J. Cardinale, University of Michigan / Cooperative Institute for Great Lakes Research; A.D. Steinman, Grand Valley State University / Annis Water Resources Institute; M.E. Ogdahl, University of Michigan / Cooperative Institute of Limnology and Ecosystems Research

A comprehensive inventory of ecosystem services across the entire Great Lakes basin is currently lacking and is needed to make informed management decisions. A greater appreciation and understanding of ecosystem services, including both use and non-use services, may have avoided misguided resource management decisions in the past that resulted in negative legacies inherited by future generations. Given the interest in ecosystem services and lack of a coherent approach to addressing this topic in the Great Lakes, a summit was convened involving 28 experts working on various aspects of ecosystem services in the Great Lakes. The invited attendees spanned a variety of social and natural sciences. Given the unique status of the Great Lakes as the world's largest collective repository of surface freshwater, and the numerous stressors threatening this valuable resource, timing was propitious to examine ecosystem services. Several themes and recommendations emerged from the summit. There was general consensus that: 1) a comprehensive inventory of ecosystem services throughout the Great Lakes is a desirable goal but would require considerable resources; 2) more spatially and temporally intensive data are needed to overcome our data gaps, but the arrangement of data networks and observatories must be well-coordinated; 3) trade-offs must be considered as part of ecosystem services analyses; and 4) formation of a Great Lakes Institute for Ecosystem Services, to provide a hub for research, meetings, and training is desirable. Several challenges emerged during the summit which will also be presented.

592 Why care about air? Deposition of air pollutants are linked to a broad range ecosystem services and their users

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Anthropogenic stressors such as pollution and climate change are driving shifts in ecosystem function and resilience in both terrestrial and aquatic systems. Scientists generally rely on biological indicators of these stressors to signal that ecosystem conditions have been altered beyond an acceptable amount. However, these biological indicators can be non-descript or non-charasmatic species that fail to spur public interest or policy consideration. Therefore, we developed the STEPS (STressor – Ecological Production function – final ecosystem Services) Framework to develop "chains" that link a change in a biological indicator to an ecosystem component that is directly used, appreciated, or valued by humans, through an ecological production function (EPF). The EPF describes distinct cause-and-effect relationships, based in scientific literature, that connect the change to the biological indicator to cascading and sequential impacts on downstream ecosystem components until a valued ecological endpoint is defined. Each ecological endpoint is linked to discrete beneficiaries, or direct users of the ecological endpoint, who are identified to evaluate those potentially impacted by the change. The framework uses a qualitative score for each link in the EPF to classify the state of the scientific knowledge. We tested the STEPS Framework in a workshop by identifying chains for four modes of response to excess nitrogen and sulfur deposition: aquatic acidification, aquatic eutrophication, terrestrial acidification, and terrestrial eutrophication. The workshop participants identified 47 biological indicators of acidification and eutrophication, and linked them to 84 ecological endpoints thorugh 183 unique EPFs. These endpoints were tied to 25 different benefiary groups, averaging 6 groups per endpoint, and created 1103 chains. Identifying the broader impacts of the stressor allows researchers, managers, and policy makers to better understand the potential impacts of change and may assist in identifying the tradeoffs of different actions. Differentiating the strength of the chains assists in prioritization of future research, potential areas of management action, and/or future valuation. The chains are also being used as a communication tools so that managers can translate the impacts of the stressor to an ecological endpoint that a beneficiary-specific audience values.

593 Determining preferences for ecosystem benefits in Great Lakes Areas of Concern from photographs posted to social media

T. Angradi, USEPA / Office of Research and Development, Mid-Continent Ecology Division; J.J. Launspach, CSRA

Relative valuation of potentially affected ecosystem benefits can increase the legitimacy and social acceptance of ecosystem restoration projects. As an alternative or supplement to traditional methods of deriving beneficiary preference, we downloaded from social media and classified »21,000 photographs taken in two Great Lakes Areas of Concern (AOC); the St. Louis River and the Milwaukee Estuary. Our motivating presumption was that the act of taking a photograph constitutes some measure of the photographer's individual preference for, or choice of, the depicted subject matter among all the other possible subject matter. Overall, 17% of photos downloaded from the photo-sharing sites Flickr, Instagram, and Panoramio depicted an ecosystem service or benefit in the AOC. Percent of images depicting a service or benefit and their subject matter varied between AOCs and among photo-sharing sites. Photos shared on Instagram were less user-gender biased than other photo-sharing sites and depicted active recreation (e.g., trail use) more frequently. Local users shared more photos depicting services or benefit than non-local users. The spatial distribution of photograph locations varied between photos depicting and not depicting a service or benefit, and identified areas within AOCs from which few photographs were shared. As a source of beneficiary preference information, we think Instagram has some advantages over the other photo-sharing sites. When combined with other information, spatially-explicit relative valuation derived from aggregate social preference can be translated into information and knowledge useful for restoration decision making.

594 Contaminant Removal by Native Mussels in the Mississippi River: An Ecosystem Service?

J.M. Archambault, W. Cope, North Carolina State University / Applied Ecology; T. Newton, USGS; H. Dunn, Ecological Specialists, Inc.

Mussels ingest contaminants as they filter water for nutritional gain. Because native freshwater mussels (Family Unionidae) comprise a large proportion of benthic biomass, and each individual filters several liters of water/day, there is potential for mussel populations to sequester large masses of contaminants. We quantified the ecological function of contaminant removal by native freshwater mussels and assessed the magnitude of their ecosystem services related to reducing contaminants for ecosystem and human well-being. The main objectives of the research were to use population estimates to calculate the total mass of contaminants sequestered by mussels and to understand the relative value of contaminant removal by native mussels (e.g., water treatment), and the cost of other important ecosystem services lost (e.g., nutrient cycling) from impacts on mussel populations (e.g., population decline). To address these questions, the three dominant species from each of two pools in the Upper Mississippi River were collected (*Amblema plicata, Fusconaia flava,* and *Lampsilis cardium* from Pool 5 and *A. plicata, Obliquaria reflexa,* and *Quadrula quadrula* from Pool 18), and soft tissues were analyzed for a suite of contaminants. An analysis of the average total metal tissue concentrations (for 22 metals) in the mussels collected, scaled up to the overall population estimates of 190 million and 212 million mussels in Pools 5 and 18 respectively, suggests that the native mussel fauna from just these two pools in the Upper Mississippi River contained a total of 18.2 tons of metals. We will also present findings from tissue analyses of organic contaminants. Our research will begin to elucidate the scale of contaminant sequestration by native mussels in a large river system, provide information to the public and stakeholders about the value of healthy mussel populations to water quality, and highlight that the beneficial reach of pollution prevention extends from our ecosystems to our faucets.

595 Creating Ecosystem Services and River Island Habitat Using Strategic Placement of Dredged Material

B. Suedel, US Army Corps of Engineers / CEERD-EPR; J. Berkowitz, USACE Engineer Research and Development Center; J. Corbino, US Army Corps of Engineers / New Orleans District

The US Army Corps of Engineers (USACE) New Orleans District utilized dredged material to nourish a sand bar that had naturally formed in the Atchafalaya River, LA. Over a 12-year period, dredged material was placed upriver of the island using shoal material removed from an adjacent Federal navigation channel during routine channel maintenance. The dredged material was dispersed downstream by river currents, thereby nourishing and self-designing the island. This project demonstrates USACE Engineering With Nature (EWN) concepts, where environmental and other benefits are being identified and quantified, enabling more sustainable delivery of economic, social and environmental benefits associated with river infrastructure. Monitoring studies were performed to determine the river hydrology necessary to create the island via transport from dredged material strategically placed upstream. This beneficial use of dredged material has created nearly 100 acres of river island habitat. Ecological surveys of the island identified over 80 species of plants and over 20 faunal species, many native to the region. The benefits being realized by the Horseshoe Bend project communicate returns on investment (i.e., benefits) supporting the implementation of EWN concepts. The creation and development of Horseshoe Bend Island has resulted in the realization of benefits ranging from the existence of additional wildlife habitat to waterborne navigation enhancements. To account for the benefits associated with this strategic placement of dredged material we documented the change in ecosystem services based on available data: 1) improve the environment or enhance ecosystem sustainability, 2) carbon sequestration, 3) nutrient sequestration, 4) research opportunities, and 5) navigation support and maintenance. These services capture a broad array of benefits associated with implementing EWN through strategic placement of dredged material. Demonstration of this approach, and fostering its integration into USACE business practices of project design, is intended both to increase project value and to more effectively manage the nation's waterways.

596 Landscape-Level Sagebrush Ecosystem Conservation Agreement: Process and Early Stage Implementation

E.J. Dorward-King, Newmont Mining Corporation / Sustainability and External Relations; J. White, Newmont Mining Corporation / Elko Land and Livestock

In August 2016 the State of Nevada (acting through the Department of Wildlife (NDOW) and the Department of Conservation and Natural Resources (DCNR)) and the U.S. Department of the Interior (acting through the Fish and Wildlife Service (FWS) and the Bureau of Land Management (BLM)) announced an agreement with Newmont Mining Corporation for a sagebrush ecosystem Conservation Framework Agreement for the stewardship of approximately 1.5 million acres of habitat in Nevada. This agreement is among the first of its kind in the United States for its scope and scale, and establishes a mutually-agreed upon framework guiding Newmont's management of sagebrush habitat under the company's stewardship. The Conservation Framework Agreement, along with a landscape-level, multi-species conservation plan, monitoring and adaptive management, a conservation bank (credit system), rangeland research, and partnership-based implementation, comprise Newmont's Sagebrush Ecosystem Conservation Program. The Agreement enables the State of Nevada to work with federal agencies and Newmont to put into practice its Conservation Credit System (CCS). The presentation will describe the stakeholder process which led to the successful signing of the agreement, highlight the key elements of the agreement framework including the incorporation of adaptive management, and describe the first projects being implemented. Among the initial projects are conservation actions to enhance riparian and mesic areas to support Greater Sage-grouse brood habitat needs and to address the expansion of invasive annual grasses as the primary threat to the sage-steppe ecosystem. Other related Newmont activities include the testing of invasive species management techniques; management to promote native sagebrush ecosystem health by increasing the density of deep-rooted perennial grasses; and implementation of practices to reduce human-induced advantages for predators of greater sage-grouse. The company also will partner with the BLM to implement sagebrush ecosystem enhancement measures on BLM-managed public lands in Nevada.

A 50-Year Retrospective of Scientific Contributions of the Duluth USEPA Water Lab to Environmental Toxicology and Chemistry

597 The Genesis and Early History of the USEPA Research Laboratory in Duluth

D.I. Mount

Among the many facets of the turbulent 1960s was a growing public awareness and outrage over the pollution of water, air, and land of the United States as a result of growing population and industrial development. Management and control of this degradation was made difficult by both limited scientific understand of how to assess and control pollution, and the relative lack of effective legislation providing authority to address such pollution. Against this backdrop, the 1961 Amendments to the Federal Water Pollution Control Act included directives to develop a research program to address water pollution and, as part of that, to construct federal research facilities to help carry out this research. In 1962, Rep. John Blatnik announced that Duluth, MN was selected as the location for one of these Federal research facilities, followed by groundbreaking in 1965, and dedication of the "National Water Quality Laboratory" in 1967. Thus began 50 years of research into the effects of human-induced pollution on fresh waters, initially under the Department of Interior and later transferred to the U.S. Environmental Protection Agency after its formation in 1970. Early research efforts centered on the development and standardization of toxicity testing methods, defining the responses of fish and invertebrates to chronic exposure to metals, and to understanding the effects of a taconite processing facility on the waters of Lake Superior. Into the 1980s, research emphasis shifted to new issues, including assessing the ecological effects of pesticides, developing methods to measure effluent toxicity and identify its sources, understanding the effects of acid rain, and developing means to predict the toxicity of organic chemicals based on their structure. This presentation reviews this early history of the Duluth laboratory from the perspective of its first director.

598 Aquatic Life Criteria: The 1985 Guidelines and Beyond

R.J. Erickson, USEPA / ORD NHEERL / Mid-Continent Ecology Division

The publication in 1985 of USEPA's "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses" culminated several years of effort by personnel from the EPA's Environmental Research Laboratories in Duluth, MN; Narragansett, RI; and Corvallis, OR to develop a more rigorous framework for the issuance of aquatic life criteria by EPA's Office of Water. This presentation will describe the genesis of these guidelines and their landmark contributions to setting water quality criteria and standards in the U.S. and elsewhere. Relative to prior criteria efforts, notable features included a formal derivation procedure to provide consistent results, concentrations for both acute and chronic effects, minimum data requirements for taxonomic coverage, species sensitivity distribution analysis, averaging periods and frequencies to fully define exposures, guidance for applying acute:chronic ratios, and a process to address the dependence of toxicity on physicochemical exposure conditions. Related guidance also addressed site-specific modifications of criteria. This presentation will also summarize subsequent and ongoing efforts in EPA's Office of Water and Office of Research and Development to further improve various aspects of criteria formulation. The contents of this abstract neither constitute nor necessarily reflect USEPA policy.

599 The incorporation of toxicity testing into the assessment and management of effluents and contaminated sediments

D.R. Mount, USEPA / ORD / NHEERL / Mid-Continent Ecology Division-Duluth, MN; G.T. Ankley, USEPA / National Health and Environmental Effects Research Laboratory; D.I. Mount; T.J. Norberg-King, USEPA / NHEERL / Mid-Continent Ecology Division

Following the initial push during the 1970's to develop numerical water quality criteria for many environmental contaminants, it became clear that the protection of surface waters from chemicals in municipal and industrial effluents would require more than just criteria for specific pollutants. To supplement chemical-specific limits, whole effluent toxicity (WET) monitoring and control was conceived, which offered three important advantages over chemical-specific limits alone: 1) toxicity testing can detect effects from unknown or unmeasured chemicals; 2) testing the toxicity of intact effluent automatically considers the aggregate effects (whether antagonistic, additive, or synergistic) of all chemicals present; and 3) effects of effluent or receiving water chemistry on chemical bioavailability was intrinsically considered. During the 1980's the Duluth laboratory invested heavily in a research program to support implementation of WET-based permitting, with the goals of 1) developing toxicity test methods that could meet the practical demands of effluent testing; 2) demonstrating the connection between effluent/ambient water toxicity and effects on instream communities; and 3) developing methods to identify the causes of effluent toxicity so that the sources of WET could be effectively controlled. Through the 1990's, a conceptually similar research program was undertaken to provide tools for contaminated sediment assessment, offering some of the same advantages as were offered by WET for toxicants in effluents – detection of unknown or unmeasured chemicals, and implicit consideration of mixtures and bioavailability. The sediment research program contained many similar elements, including the development of methods for measuring sediment toxicity, and methods to identify the causes of toxicity in toxic sediments. Additional research was focused on methods to develop numerical criteria for specific sediment toxicants that could address the considerable challenge of addressing factors affecting the bioavailability of sediment contaminants. This presentation provides an overview of the practical needs, technical challenges, and scientific accomplishments addressed by the effluent and sediment research programs. This abstract does not necessarily reflect USEPA policy.

600 Understanding and Predicting Metal Bioavailability

W.J. Adams, Red Cap Consulting

This presentation will be a review of the development of the science of metal bioavailability to aquatic organisms and the role that the scientists at USEPA's Office of Research and Development Duluth Laboratory played in developing this crucial information. In developing water and sediment quality criteria for metals, it is essential that bioavailability be a prime consideration. The literature is replete with observations that total metals do not correlate with toxicity observations. The key to making accurate predictions or observations of toxicity is to understand the role that water and sediment chemistry play in controlling speciation and bioavailability of metals. Work at the Duluth Laboratory on the development of water quality criteria and bioavailability of metals goes back to the early 1960s where the effects of hardness and pH on zinc toxicity to fish were examined (Mount, D.I., 1964). Andrew (1974) reported on correlations of copper (II) toxicity with ionic activities in natural waters. A sentinel paper on bioavailability of copper to fathead minnows was published by Erickson et al. (1996) which laid the groundwork for the development of the biotic ligand model for copper and implicit assessment of bioavailability. Research on the role of acid volatile sulfide in controlling metal bioavailability in sediments began in 1991 (Ankley et al. and Carlson et al., 1996) and culminated with a classic publication on the Technical Basis and Proposal for Deriving Sediment Quality Criteria for Metals. More than 210 publications have resulted from research on metals at the Duluth Laboratory. The breadth of this research will be reviewed.

601 How Our Understanding of Bioaccumulation was Catalyzed by the USEPA Duluth Laboratory

D.L. Swackhamer, *University of Minnesota*

The accumulation of hydrophobic organic compounds is now well documented and its mechanism described. It is important to honor how much of that understanding was done by scientists with the USEPA Duluth Lab. The first report of polychlorinated biphenyls (PCBs) accumulated by top predator fish was in Lake Michigan by USEPA former scientist and laboratory director Gil Veith when he was a graduate student. This led to a focus on this issue as both an ecological and human health threat and resulted in research by a series of excellent USEPA scientists that provided an understanding and a modeling framework of bioaccumulation. This presentation will provide that important history and put it in context of current research needs and challenges.

602 Development of physiologically based toxicokinetic (PBTK) models for fish: Confessions of a former fish physiologist

J.W. Nichols, *USEPA / ORD NHEERL / Mid-Continent Ecology Division*

In ecotoxicology, as in toxicology generally, the fundamental paradigm used to describe chemical interactions with biological systems is the dose-response relationship. Depending on the chemical, however, the most relevant expression of dose may any one of several metrics related of the chemical concentration time-course in the environment or within the organism itself. Moreover, a need exists to predict appropriate dose metrics for 1000s of untested chemicals and species in a manner that can be related to exposure conditions of interest. To address these challenges, researchers at the EPA laboratory in Duluth, working in collaboration with other scientists, have adopted methods from fish physiology to develop a mechanistic understanding of processes that control chemical uptake and accumulation in fish. Early research demonstrated that temperature and dissolved O_2 impact chemical uptake from water by influencing gill ventilation rate. Additional work showed that branchial uptake and elimination vary predictably with chemical log K_{ow}, and that these relationships can be described using a mechanistic model for counter-current exchange at fish gills. Early PBTK models employed this gill sub-model to describe uptake and accumulation of chemicals that diffuse passively across cell membranes and partition non-specifically to tissue lipids. These models have since evolved to include descriptions of chemical flux across the skin and gut, and biotransformation in the liver. Subsequent work has shown that PBTK models may be used to extrapolate kinetic observations among fish species. Importantly, these advances have provided an improved understanding of conditions under which model complexity collapses and kinetic behavior can be adequately simulated using very simple descriptions. Ongoing work is focused on chemicals that exhibit more complex behaviors, including specific binding to plasma proteins and active secretion to urine. Descriptive compartmental and non-compartmental modeling approaches are being used with increasing frequency to quantify these behaviors and inform future development of PBTK models. The continuing goal of this effort is to develop mechanistic models of appropriate complexity to address the evolving needs of aquatic toxicologists and environmental risk assessors. The contents of this abstract neither constitute nor necessarily reflect USEPA policy.

603 Predicting Toxicity from Chemical Structure: The Narcosis Story

D.M. Di Toro, *University of Delaware / Civil and Environmental Engineering*

The development and application of methods of predicting toxicity due to narcosis and other modes of action was, and continues to be, an important component of the work at the Duluth USEPA Laboratory. The modern quest for methods that can predict toxicity using only molecular structure is rooted in the discovery in the mid 1980's by Gill Veith and Hans Konemann of the inverse relationship between the LC50 and Kow for narcotic chemicals. A contemporary finding by Steven Broderius was that the toxicity of a binary mixture of narcotic chemicals could be predicted by adding the LC50 normalized concentrations, i.e. using toxic units. These early discoveries and testing and development of the fathead minnow data base laid the groundwork for the modern developments of QSARs. These insights, and the idea that the specific critical body burden of the narcotic chemical can explain the inverse LC50 – Kow relationship, form the basis of more modern toxicity models. For example the Target Lipid Model utilizes the species specific critical body burden and the additional idea that the lipid-water partition coefficient is species independent and depends only of the chemical properties of the narcotic. This and other modern formulations point to the future development of aquatic criteria for which the molecular structure is the only chemical information needed. They can be structured using the framework presented in the Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses, another seminal EPA accomplishment in which the Duluth Lab was central to its development. And they can be applied to mixtures of compounds with the same mode of action.

604 Ecotoxicology in the 21st Century—AOPs, HTT and other Acronyms

D.L. Villeneuve, *USEPA / National Health and Environmental Effects Research Laboratory*

In 2007 an influential National Research Council report outlined the limitations of the traditional, empirically-driven, whole organism approach to guideline toxicity testing. The committee laid out a vision and strategy for toxicity testing in the 21st century that would take advantage of decades of advances in biology and technology to evaluate chemical safety more rapidly and cost effectively. At the heart of this approach was the greater application of mechanistic or pathway-based data, such as those which could be obtained through high throughput, in vitro, chemical testing, to predict toxicological outcomes without directly observing them in whole animal toxicity tests. As a response to this strategy, a working group at USEPA Duluth proposed the adverse outcome pathway (AOP) concept as an important component of an overall strategy for toxicity testing in the 21st century. While the AOP concept was not new, having deep roots in QSAR and biomarker-related research in the field of ecotoxicology, it was recognized as having a valuable and important role to play in this new vision for toxicity testing. As a result, the AOP concept has taken on prominence in ecotoxicology and human health toxicology alike. What was previously a cogitative concept has developed into a formal framework for organizing toxicological knowledge and evidence and a driving force in the USEPA Duluth and broader Office of Research and Development research portfolio. USEPA Duluth remains at the forefront of the development and application of the framework, providing some of the earliest examples of formal AOP description, development of quantitative AOPs, and construction of AOP networks. Just as importantly, it remains committed to demonstrating the practical application of AOPs to enhance interpretation of HTT (high throughput toxicology) data streams, development of IATA (integrated approaches to testing and assessment), cross-species extrapolation (e.g., SeqAPASS), and monitoring of the environment (e.g., through the use of EARs [exposure-activity ratios] and EDA [effects-directed analysis]). Building on 50 years of scientific contributions, USEPA Duluth continues its mission to protect human health and the environment. The contents of this abstract neither constitute, nor necessarily reflect, USEPA policy.

Perfluoroalkyl Acids (PFASs) – Historical and Current State of Affairs (Minnesota Emphasis)

605 Perfluoroalkyl Substances (PFAS) in Minnesota - Investigating a Multi-Site Legacy Megaplume and Hunting for Other Sources

V. Yingling, *Minnesota Department of Health / Environmental Health; J. Wallerstedt, Minnesota Pollution Control Agency / Remediation Division; S. Thomas, AMEC Foster Wheeler*

Since 2004, Minnesota has been investigating PFAS contamination at a manufacturing facility and at its three main disposal sites in Washington County, on the east side of the Minneapolis-St. Paul metropolitan area.

The co-mingled plumes emerging from these sites have created a "mega-plume" that affects the groundwater in an area over 130 square miles, contaminating eight municipal drinking water supplies and over 1,800 private wells. Early investigations focused on groundwater transport and the influence of bedrock structures, but our increased understanding of the role of surface water and stormwater PFAS transport has widened the investigation to areas previously not suspected to be impacted. This has important implications for investigations at other PFAS sites, where these highly soluble and persistent chemicals may follow transport pathways not typically of concern for most organic environmental contaminants. Minnesota's early investigations of PFAS also led to state-wide efforts to identify and evaluate other sources and impacts. This talk will briefly touch on statewide PFAS investigations, the major sources identified - such as aqueous film forming foam (AFFF) at fire training and/or fighting sites, plating facilities, wastewater treatment plants - and efforts to inventory and prioritize potential PFAS sources for further evaluation.

606 Incorporating Indirect Exposure Pathways - Derivation of Health-based Water Guidance for PFOA and PFOS Using an Excel-based Toxicokinetic (TK) Model

H.M. Goeden, Minnesota Department of Health / Environmental Health; C. Greene, Minnesta Department of Health; J. Jacobus, Minnesota Department of Health / Health Risk Assessment

Perfluorinated compounds are ubiquitous environmental contaminants and have been widely detected in environmental media, humans and wildlife. In May of 2017, the Minnesota Department of Health (MDH) released revised noncancer health-based water guidance (nHBG) for PFOS and PFOA. Traditionally, noncancer health-based water guidance values are calculated using a simple equation containing a reference dose, a relative source contribution factor, and water intake rate. However, due to the bioaccumulative nature of PFOS and PFOA it became clear that traditional nHBG derivation methods would be inadequate to address all drinking water related exposure pathways of concern. Research has shown that breastmilk can be a major source of exposure, resulting in infant serum concentrations that are higher than maternal concentrations. Although exposures during infancy are short-term, this life stage is of particular concern because (1) infants consume a much greater volume of liquid per unit body weight than older children and adults; and (2) due to the long elimination half-lives, the body burden instilled in infancy may take years to eliminate. To address these concerns, MDH developed a novel Excel-based TK model tailored to the physical-chemical properties, exposure parameters, and human transfer coefficients especially critical for evaluating early life exposures. Two reasonable maximum exposure scenarios were evaluated: 1) an infant exclusively fed with formula reconstituted with contaminated water starting at birth, followed by a lifetime of drinking contaminated water; and 2) an infant exclusively breastfed for 12 months, followed by a lifetime of drinking contaminated water. In both scenarios, infants began life with a pre-existing body burden through placental transfer from their mother at steady-state conditions. Due to chronic bioaccumulation in the mother and subsequent transfer to breastmilk, breastfed infants were the most heavily exposed, and predicted serum levels early in life exceeded steady state levels. To ensure protection of all segments of the population MDH's revised nHBG were set at levels intended to be protective of breastfed infants.

607 Longitudinal Biomonitoring for PFAS in a Minnesota Community with Past Drinking Water Exposures

J. Nelson, Minnesota Department of Health; C. Rosebush, University of Minnesota; C.A. Huset, Minnesota Department of Health / Organic Chemistry Unit; J. Johnson, Minnesota Department of Health

Background: The Minnesota Department of Health has conducted longitudinal biomonitoring to measure PFAS in residents of Washington County, Minnesota. In 2004, state agencies discovered PFAS contamination in area groundwater and private drinking water wells. In response, public health interventions were initiated including water treatment by granular activated carbon. The purpose of the biomonitoring projects was to determine community exposures and check effectiveness of the interventions at reducing drinking water exposures to PFAS. Methods: Biomonitoring projects conducted in 2008, 2010 and 2014 measured seven PFAS chemicals in 149 longer-term residents of the area. In addition, the 2014 project measured PFAS in a group of 156 newer residents in the area who moved after public interventions were made to reduce PFAS levels in drinking water. The MDH Public Health Laboratory analyzed serum samples for PFAS using liquid chromatography tandem mass spectrometry (LC/MS/MS). Results: Serum concentrations of the three most common PFAS (PFOS, PFOA and PFHxS) declined by 34-59% between 2008 and 2014. These declines are similar to what would be expected based on results from other exposed populations and on half-life estimates. Serum concentrations in the group of newer residents were similar to background levels observed in NHANES. These findings indicate that interventions to reduce drinking water exposure to PFAS in this community in Minnesota were effective.

608 The Perfluoroalkyl Substances (PFAS) in Homes and Gardens Study: Implications for Human Exposure

D. Scher, Minnesota Department of Health; J.E. Kelly, Minnesota Department of Health / Environmental Health; C.A. Huset, Minnesota Department of Health / Organic Chemistry; R. Hoffbeck, V. Yingling, R. Messing, Minnesota Department of Health

Background: Since 2004, Minnesota has responded to PFAS contamination in groundwater serving as the drinking water supply for several Twin Cities communities. Granular activated carbon (GAC) filtration systems were installed to treat public and private wells with PFAS levels exceeding MN Department of Health (MDH) guidelines. Although drinking water exposures were addressed, past use of contaminated water to irrigate lawns and gardens may have left residual soil contamination that contributes to ongoing exposure. Further, water used for irrigation may still contain PFAS, since home GAC treatment units may not treat exterior taps and some shorter-chain PFASs are found to "break through" GAC filters under certain conditions. In 2010, MDH investigated whether past or ongoing PFAS water contamination results in elevated concentrations of PFAS in soil and home-grown produce. Methods: Exterior tap water, garden soil, and produce samples were collected at homes with a history of water contamination. Samples were analyzed by MDH for several PFAS using LC/MS/MS. Results: In exterior tap water, perfluorobutanoic acid (PFBA) was found most often and at higher concentrations compared to other PFAS. In garden soil, PFBA, perfluorooctane sulfonate (PFOS), and perfluorooctanoic acid (PFOA) were found in all samples and at the highest concentrations. In produce, PFBA was detected most often and at higher concentrations compared to other PFAS. Significant determinants of PFBA concentration in produce were the amount of PFBA applied to the garden via watering and produce type. Conclusions: This study demonstrates PFAS entry into the terrestrial food chain. Short-chain PFAS in water influenced produce levels more than long-chain PFAS in soil. The results are consistent with experimental studies demonstrating preferential uptake of short-chain PFAS by edible plants; bioaccumulation of short-chain PFAS in plants; and differences in PFAS accumulation by produce type/vegetative structure. Although the general population is mainly exposed through diet, mechanisms of PFAS food contamination are still poorly understood. Data are particularly lacking for shorter-chain PFAS even though they have the highest potential to contaminate water and bioaccumulate in crops. These findings are globally relevant, as short-chain PFAS serve as commercial substitutes for longer-chain compounds and are increasingly detected in water due to their relatively high solubility and mobility.

609 PFOS in Minnesota Fish: Data and Risk Assessment Considerations for Fish Consumption Advice

P. McCann, Minnesota Department of Health / Environmental Health; B.A. Monson, S. Streets, Minnesota Pollution Control Agency / Environmental Analysis and Outcomes

Since 2004, Minnesota has tested fish for perfluorochemicals (PFAS) starting with six bass from Pool 2 of the Mississippi River. As of the most recent collections in 2016, fish from seven rivers and over 150 lakes have been analyzed. Most of these waters have only been tested once. Seven lakes and Pool 2 of the Mississippi River are listed as impaired, which has been the basis for continued retesting in those waters. Four percent of lakes tested have data from within the past 5 years. Perfluorooctane sulfonate (PFOS) is the PFAS that accumulates to the highest levels in fish. Declines in fish PFOS levels over time are evident in the majority of waters that have more than one year of data, with some showing steep declines. Declines in some of these waters are likely related to source elimination or structural changes in the watershed. Explanations have not been identified for some observed declines. PFOS levels in fish from some waters increased between sampling events. Spatial variation in PFOS levels were also observed within and among species collected at different locations within a waterbody. Implications for fish consumption advice from variation in fish PFOS concentrations and potential changes in the risk assessment for PFOS will be illustrated. The half–life of PFOS in fish is not well established. Shorter half-lives than most fish contaminants causing advisories have been suggested, with half-lives ranging from around one month to one year reported. This raises questions about including multiple years of data and data from past sampling when determining fish consumption advice. Minnesota Department of Health fish consumption guidelines have used an "MDH-reference dose" derived in 2008 as the basis for advice in Minnesota. The U.S. Environmental Protection Agency released a health advisory, and reference dose (RfD) in 2016, to reflect the latest scientific evidence about the risk posed by PFOS. This EPA RfD is lower than the one currently used in Minnesota, and if adopted, may result in adjustments to the advisories.

610 Source of PFOS impairment associated with novel route of environmental release

S. Streets, Minnesota Pollution Control Agency / Environmental Analysis & Outcome; F. Campbell, S. Sokola, T. Maurice, C. Biglow, J. Elling, Minnesota Pollution Control Agency

In 2004, investigators discovered that fish in Lake Calhoun were contaminated with perfluorooctane sulfonate (PFOS). At that time, this discovery was unexpected, as there was no known direct source of PFOS to Lake Calhoun. Fish tissue concentrations were high enough to warrant a 1-meal-per-month fish consumption advisory, which led to the declaration of an impairment in the lake. Beginning in 2007, MPCA conducted a series of investigative studies to determine the source of PFOS to the lake. Stormwater samples on the west side of the lake showed very high concentrations of PFOS. Stormwater sampling continued, and moved progressively westward from the lake. Higher concentrations were detected with each subsequent sampling event. A closer look at the nearby businesses revealed the presence of a chrome plating operation within a ½ block of the sample location with the highest stormwater PFOS concentration. During a surprise inspection of the plating facility, inspectors determinedthat no illicit stormwater connection existed. Inspectors discovered that plating lines inside the building were vented to the roof. Snow samples collected around the roof vents during the inspection indicated very high concentrations of PFOS and other PFASs and chromium. Subsequent confirmation samples were taken, including snow on the roof, water from the roof drains, and stormwater runoff. All samples indicated high concentrations of PFOS. To our knowledge, this type of release has not been described in current literature.

611 Mixtures of poly- and perfluoroalkyl substances (PFASs) reduce growth and delay development of a larval amphibian

M. Gannon, Purdue University / Forestry and Natural Resources; M. Chislock, Purdue University; B. Tornabene, J. Hoverman, Purdue University / Forestry and Natural Resources; M.S. Sepulveda, Purdue University / Forestry and Natural Resources and School of Health Sciences; S. Gray, S. Bauer, Purdue University

Abstract Poly- and perfluoroalkyl substances (PFASs) have recently been detected in drinking water sources increasing the awareness of potential adverse effects of PFASs on human health and the environment. Acute toxicities of individual PFASs are well-studied and we are rapidly gaining an understanding of sublethal effects such as low birth weights in humans and thyroid disruption across many taxa. However, effects of PFAS mixtures which are a common occurrence in nature remain largely unexplored. To address this gap, we compared the effects of individual and mixtures of PFASs on growth and development of larval American bullfrogs (*Rana catesbeiana*). We tested environmentally relevant concentrations of two of the most prevalent and persistent PFASs, Perfluorooctanesulfonic acid (PFOS) and Perfluorooctanoic acid (PFOA) at 0.1 and 0.2% of the LC50 values (144 and 288 ppb, respectively). Exposure to only PFOS, and mixtures with PFOS, delayed development and reduced growth of tadpoles by > 30% at the conclusion of our 74-d exposure period. Though the most deleterious effects were observed in mixtures of PFASs with PFOS, the severity of delayed development and growth was independent of varying concentrations. These results suggest that PFAS mixtures have synergistic sublethal effects on larval amphibians at environmentally relevant concentrations. Though recent studies have revealed the threat that single PFASs have on human health as well as the environment, this study is of the first to explore the effects of PFAS mixtures in nature which is a more realistic circumstance. Due to the negative ecological consequences highlighted in this experiment PFAS mixtures warrant further investigation in future environmental assessments.

612 How Did We Get Here? A Retrospective Look at Emerging Contaminant Regulatory Trends and How They Infer Future Direction

S. Thomas, H. Albertus-Benham, Amec Foster Wheeler Environment & Infrastructure

Emerging contaminants have been defined by the USEPA as those chemicals or materials that have a perceived, potential, or real threat to human health or the environment, lack published health standards, and/or new source or pathway information has become available. Emerging contaminant programs were developed nearly a decade ago by the USEPA, the United States Geological Society (USGS) as well as the Department of Defense (DoD). Despite being around for almost 10 years, emerging contaminants have not had elevated and extensive visibility until very recently with the momentum of contaminants such as 1,4-dioxane and Per- and Polyfluoroalkyl substances (PFASs). If no promulgated health criteria are available and science surrounding these contaminants continue to evolve, how have they become such hot button liability issues across the US and globally? What key factors led us to where we are today and how will these factors influence the regulatory direction of future emerging contaminants? Key factors that have led us to where we are today. Using PFASs as an example, the history and timeline of regulatory, social, and litigious activity will be presented as well as the associated research progress, trends, and challenges that are arising as regulations continue to develop. An overview of the various international, federal and state emerging contaminants programs will be presented and the Safe Drinking Water Act (SDWA), the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), Toxic Substance Control Act (TSCA), and the National Health and Nutrition Examination Survey (NHANES) will be discussed in relation to their part in elevating PFASs to the priority status they currently have today. What factors influence the direction of future emerging contaminants? Reactions to PFASs from government, industrial sector, and water authorities will be discussed. Proactive legislation at various state, federal, and international levels that ensures lines of liability are clearly defined will be presented. Forward-thinking programmatic infrastructure to support regulatory action will be highlighted. What to take-away? Whether in industry, government, or private sector, emerging contaminants will continue to be defined for years to come and a thoughtful evaluation of historical activities will successfully enable any organization to better prepare for and proactively manage the future evolution and liability of these dynamic group of chemicals.

Incorporating Environmental Toxicology Into the Classroom

613 Explorations in Environmental Toxicology: A Summer Institute for High School Students

F. Lambert, University of Florida / Center for Environmental and Human Toxicology; L. Lundgren, University of Florida / School of Teaching and Learning; J. Bokor, M. Koroly, University of Florida / Center for Precollegiate Education and Training

Explorations in Environmental Toxicology is a two-week summer institute held at the University of Florida (UF) for junior and senior high school students. University researchers, government workers, and local industry professionals facilitate hands-on learning experiences to introduce students to environmental toxicology concepts and careers. These professionals engage students in unique learning experiences that explore the theories, technologies, and current research topics within environmental toxicology. Activities include molecular biology bench work, field sampling in local environments, engaging lectures, and field trips to areas of interest. Students apply learned concepts and techniques by producing research proposals regarding place-based environmental toxicology issues. The proposals are presented to fellow students, parents, and community members in a 'mini-conference' setting at the end of the program. The institute provides a unique opportunity to acquaint high school students with the multi-faceted and interdisciplinary field of toxicology, providing an academic and career path that may not be covered in traditional school settings.

614 Out of the classroom and into a Superfund Site? Using real problems and real people to teach elements of toxicology

M. Borrello, Alma College

There is a large gap between what we know about how students learn science and math, and how we teach science and math at the undergraduate level. Exacerbating this gap are stressors such as, limited contact time, heavy content requirements, instructors whose primary duties are not teaching, and a physical and conceptual environment not conducive to creative thinking. Fortunately, toxicology presents an ideal framework from which to stimulate interest in not only the disciplines that make it up, but science in general. Concepts related to health and risk are not merely limited to the science classroom, but are in everyday life where students can find connections in and out of the classroom. It is there where we can begin to close the gap between teaching and learning. For example, teaching from an epistemological and methodological perspective instead of one that is purely content-driven, may open pathways of learning that are richer and more salient. Project-based courses that link community problems with student expertise bring a real-world element that links theory and practice in a unique way. For the past twenty years, Alma College has utilized a local Superfund site and Community Advisory Group (CAG) as a venue to utilize new-found knowledge in addressing important human and environmental health problems. It connects students with the problem but also with those most affected by the outcome of their interactions. It is also a good way to pair students with experts in the field. Assessment results show students take much more away from their experience than content knowledge.

615 Quantifying mine soil toxicity by undergraduates in a research course

K. Sweat, P. Marashall, J. Foltz-Sweat, T. Cahill, J. Broatch, Arizona State University West Campus / School of Mathematical and Natural Sciences

In the Spring of 2017, a course based undergraduate research experience (CURE) was developed (LSC 388: Vegetative Remediation of Mine Sites) in the School of Mathematical and Natural Sciences at Arizona State University that explored the use of plants to remediate soils contaminated with mine waste. The course introduced students to the concept of phytoremediation, and then facilitated groups of students designing and executing their own research protocols. Given the length of time available (one semester), students were encouraged to look at germination toxicity from common mine soil contaminants to native plant species. Although some groups went beyond this, all of the student groups were successful in designing and executing a research protocol that quantified metal toxicity to seed germination or vegetative propagule establishment. Quantitative techniques were utilized in the research, including techniques previously unknown to the instructor. The course is an effective way to introduce quantitative analysis and other important aspects of research to undergraduate students in a classroom environment. This course is being developed as a part of a series of interdisciplinary Course Based Undergraduate research experiences, designed around a common course outline and learning objectives. Pedagogical tools and strategies to develop your own CURE and incorporate this curriculum will be presented.

616 Place-Based Education and Environmental Toxicology

D.A. Kimberly, Westminster College / Biology

Place-Based Education (PBE) is an approach to learning that takes advantage of local and regional geography to create immersive, authentic, and relevant experiences for students. Ultimately, the goals of PBE are to increase student and teacher engagement, improve academic outcomes, and positively impact local communities. Quantitative support is growing amongst many college and university educators for a hypothesis that suggests students involved in curricular and co-curricular programs that emphasize place-based learning have better overall retention and graduation rates (better than 90% in many programs), they are successful at finding jobs and/or being admitted to graduate school after graduation, and they feel deeply connected to their surrounding community. Many fields, including Environmental Toxicology, are prime subjects for PBE consideration. At Westminster College (private, undergraduate) in Salt Lake City, UT, Environmental Toxicology is taught bi-annually, with the most recent iteration being held this past spring semester. To understand the effects of a directed PBE approach on learning outcomes in Environmental Toxicology, the principles therein where applied to the Spring 2017 version of the course. I operated under the hypothesis that learning assessment benchmarks would be higher for students exposed to PBE than those students from previous iterations. Larger-scale PBE activities included superfund site visits followed by land-use surveys, research on the source, exposure, and effect of metals in local waterways, and conducting mock-environmental risk assessments in culturally, economically, and environmentally sensitive areas. Learning assessments were conducted prior to the beginning of the course (diagnostic), at periodic times throughout the semester (interim), and at the end of the semester (summative). Preliminary data suggests that interim and summative benchmarks for Spring 2017 were on average 8% (+/- 1%) higher than those of students in past iterations. This constitutes an increase of almost an entire letter grade, when using standard scoring. One of the campus wide learning goals at Westminster College is to help develop highly informed and engaged citizens. PBE is a useful framework by which to reach that important goal. While more research into PBE approaches and their advantages is needed, early signs indicate it is beneficial for student and faculty learners as well as local communities at large.

617 Incorporating envir political scientists to environmental issues

S. Nutile, Penn State Univ. / The Behrend College / Biology

As our scientific understanding of how humans affect and interact with their environment continues to advance, understanding of these issues within the political sphere is moving in the opposite direction. Political decisions regarding environmental issues are made or informed by individuals lacking the knowledge to interpret primary scientific literature for themselves, requiring potentially bias input from outside sources. Therefore, decisions surrounding these issues become hindered by the rampant spread of misinformation and emotional reactions elicited in voters by those in power. While emotion may never be removed from political decisions, the spread of misinformation through an inability to interpret scientific information threatens to destroy our way of life, as we ignore scientific evidence questioning the environmental practices upon which our society functions. As scientists and educators, we have an opportunity and responsibility to arm future generations of students interested in pursing political careers with the tools to dissect scientific information for themselves and approach these issues with a healthy skepticism. This goal can be accomplished through the development of environmental science courses aimed at those students pursuing careers in political science. The focus of this presentation will be on how to structure such a course to teach and evaluate the knowledge of the students in a way that prepares them for the challenges they will face in politics. Lectures designed to present the scientific principles behind current politically charged environmental issues offers an unbiased look at how environmental research is conducted and the facts that come from such studies. These lectures give students the tools to understand why these issues are of concern and the facts emerging from scientific research. Evaluations take the form of essays challenging the students to formulate and defend an opinion using evidence from the literature, providing them an opportunity to put what they have learned into practice. Such a course is aimed at providing students with the knowledge to correctly interpret and evaluate current and future environmental issues that will inevitably be of concern as the human population continually increases and our influence on the environment continues to expand.

618 Incorporating primary literature into the toxicology classroom

A.D. Harwood, Alma College / Environmental Studies / Biology

There are several challenges associated with teaching undergraduate students how to properly interpret and apply primary literature to topics discussed in the classroom. These problems are often exacerbated with complex topics with which the students have limited experience and exposure (e.g. environmental toxicology). This presentation will discuss strategies used to incorporate these materials into various classrooms at multiple course levels from introductory to upper level. These techniques advance understanding as the student progresses from introductory environmental science to advanced toxicology topics courses. In introductory courses students are taught to identify primary literature and learn to interpret key components from material, these materials are paired with layperson articles on similar topics. Ultimately in advanced courses, students can learn concepts from the primary literature directly and integrate these materials in the form of literature reviews, research proposals, and their own primary research. Although the current case studies are implemented in smaller classrooms (10-35 students), approaches for implementing these projects in larger courses will also be examined.

619 Ecotox in Excel: A tool to improve quantitative literacy

W.A. Wilson, Illinois Wesleyan University / Environmental Studies

Like most science, environmental toxicology is communicated in quantitative terms. A well-known example is the dose-response curve which links two sets of numbers (dose and response) together in a way that allows predictions to be made. Unfortunately, to many students and citizens, this quantitative language is opaque. For instance, while a dose-response curve might be understood by an undergraduate student on an intuitive level, most will struggle to provide an adequate description of what they are seeing. Students are also challenged to use the same curve to make predictions or compare it to a comparable curve. This difficulty in accessing quantitative information is not limited to dose-response curves. One tool that can be used to improve quantitative literacy is to make use of a spreadsheet program such as Microsoft Excel. Spreadsheet programs have become ubiquitous additions to starting computer software packages and are available to all students who have adequate computer access. Modules utilizing a spreadsheet program can teach concepts in quantitative and statistical literacy, while utilizing published data from the environmental toxicology literature. Using published data allows the modules to incorporate aspects of discussion as students analyze the data and the process that produced it. Implementation of prototype modules in multiple undergraduate courses has shown some promising results in improving quantitative literacy. This implementation has also demonstrated that these modules can be useful at different levels of student education. Sample modules will be presented.

620 Using Individual Development Plans (IDPs) to Guide Student Professional Development Training in an Environmental Toxicology Curriculum

J.E. Canas-Carrell, Texas Tech University / Environmental Toxicology

In addition to receiving training to become independent scientists, students should receive concurrent professional development. Numerous funding agencies, including the National Science Foundation and National Institutes of Health, are beginning to require that all graduate students and postdocs receive formal professional development training and mentoring through the use of individual development plans (IDPs). A course focused on professional development was created and has been incorporated as a required course for all incoming graduate students. The course is centered on the use and completion of the Individual Development Plan (MyIDP) online software provided by Science Careers. The IDP process will be discussed in detail. As a part of this process, students must complete informational interviews and then present on those interviews in class. These interviews provide an excellent opportunity for discussion of career paths in environmental toxicology. Information provided in this presentation can be used to implement professional development mentoring and training at the individual student level to a whole class level.

Improving the Environmental Assessment of Complex Composition Substances and Mixtures for Chemicals Management

621 Ecotoxicological Risk of Pollutants in Iberian Rivers

M. Kuzmanovic, IDAEA CSIC; D. Barcelo, IIQAB-CSIC / Environmental Chemistry; A. Ginebreda, CSIC - Spanish National Research Council / Enviromental Sciences

There is a concern about a large number of chemicals released into the environment and their potential risk for aquatic species. However, this task is not so straightforward since in the environment chemicals occur in complex mixtures rather than as single substances and they usually co-occur with other stressors. The aim of this study is to identify the major drivers of ecotoxicological risk and to provide the evidence of the effects in ecosystems caused by chemicals in Spanish rivers. Four rivers of Iberian Peninsula were used as case studies; namely Llobregat, Ebro, Júcar, and Guadalquivir. More than 200 organic pollutants and metals were used in this study. Metals and pesticides (e.g. organophosphate insecticides) were of highest concern in the area. Emerging contaminants, such as pharmaceuticals (e.g. sertraline or losartan) or biocides (triclosan) were contributors to the chronic risk. The mixtures of organic compounds and metals posed an acute risk at 42% and 45% of sites, respectively. The chronic risk was present at all sites. The risk posed by the Water Framework Directive priority pollutants was significant and those compounds were among the highest contributors to the risk. However, we found that banned pesticides and emerging contaminants significantly contributed to the risk. We used different macroinvertebrate based indicators to find the link between chemical pollution and ecosystems changes. We found a significant relationship between pesticides toxicity gradient and a decrease of SPEARindex the stressor-specific indicator for pesticide pollution. Furthermore, we used the functional traits of macroinvertebrate communities to find the evidence of pesticides toxicity and urban-related multiple stressors in studied rivers. It was identified that multiple stressors were present at 50% of the sampling sites, mostly in urban areas. There was a significant difference between communities exposed to pesticides and those exposed to urban-related multiple stressors. The functional diversity of assemblages at urban sites was low, suggesting the functional homogenization of assemblages in urban areas. Acknowledgements This study has been financially supported by the EU through the FP7 project GLOBAQUA (Grant Agreement No. 603629), the Spanish Ministry of Economy and Competitiveness [SCARCECSD2009-00065] and by the Generalitat de Catalunya (Consolidated Research Groups: 2014 SGR 418−Water and Soil Quality Unit and 2014 SGR 291−ICRA.

622 Characterization of Gases and Aerosols in Flowback Emissions at Hydraulic Fracturing Operations in the Cardium Formation in Central Alberta

M.L. Dumanski, Alberta Energy Regulator / Science and Evaluation Branch

Air quality impacts from energy resource development can directly and indirectly affect human and environmental health. Growing concerns over greenhouse gas emissions and climate change, noxious odours and decreased quality of life, deposition of contaminants to the environment, and direct impacts to human and environmental health from decreased air quality have become drivers in the management and development of energy sector resources. The flowback emission project presented here was initiated through recommendations from a Recurrent Human Health Complaint Assessment conducted by the Alberta Energy Regulator to better understand the cause of frequent human health and odour complaints in the vicinity of hydraulic fracturing operations in the Lochend and Didsbury areas. The report identified that chemical type, concentration, volumes released, and duration of emissions from flowback emissions ultimately combusted at the flare and released to that ambient environment from hydraulic fracturing operations are not well understood. This presentation focuses on the research study undertaken by the AER and Alberta Health and will describe the; Analysis of data submissions to Frackfocus, pre-flare stream sampling methods, sampling design, and preliminary results of characterization of gases and aerosols in flowback emissions from hydraulically fractured wells. The foundational data generated by this study is intended to support regulators, policy makers, researchers, and industry and public stakeholders in making risk based decisions during the lifecycle of hydraulic fracturing operations. This could apply to decisions around human and environmental health risks, policy direction, regulatory requirements, future research needs, technical limitations, monitoring requirements, and risk management and mitigation strategies at hydraulic fracturing operations.

623 Application of PRSTM, DGT and CaCl2 as tools to approximate plant accumulation and toxicity to nickel and cobalt alone and in a mixture

A.E. Laird, University of Guelph / School of Environmental Sciences; S. Siciliano, University of Saskatchewan / Soil Science; B.A. Hale, University of Guelph / School of Environmental Sciences

Metal contamination in soils most often occurs as mixtures of more than one metal. Although there have been numerous studies accumulation and toxicity of single metals, implications of multiple metals of concern remains largely unknown. Tools for risk assessment of mixtures of metals in soils are lacking. One possible tool is the use of ion exchange resins as proxies for plant accumulation of metals and growth. Plant root simulator (PRS^{TM}) probes have their origins in testing for soil nutrient bioavailability, but have been adapted for use in metal bioavailability. Diffusive gradients in thin film (DGT) were originally created for measuring bioavailability of metals in water, but were soon adapted for use in sediments and then soil. The application of these methods to metals in soil is not yet universal, and the implications of the multiple metals for these tools are yet to be investigated. If these tools are acceptable for this application, they may be applied as a screening tool for use in risk assessment when more than one metal of concern is present. To this end, a fixed ratio ray design was used to compare uptake and toxicity in barley to Ni and Co as singles and in a mixture that included Ni, Cu and Co. Plant accumulation and response were compared to PRS^{TM}, DGT, $CaCl_2$ extraction and pseudo total metal concentrations. Single metals were more bioavailable than in the mixture at low concentrations for both Ni and Co. However at higher concentrations, Ni became more bioavailable in the mixtures. The range of concentrations studied for Co was lower, so this effect may not have been captured if it were present. Plant accumulation was most closely mirrored by PRS^{TM} and $CaCl_2$, while DGT more closely mirrored plant toxicity as represented by growth parameters for both metals. These results illustrate a notable concentration dependence on metal bioavailability in mixtures. At low concentrations, metals are adsorbed to specific binding sites on soil and are not removed by the presence of additional cations. However, at higher concentrations adsorption is largely attributed to exchange reactions. Furthermore, plant uptake is metal specific at lower concentrations, while, uptake at higher concentrations are not. PRS^{TM} and DGT show promise as tools to assess the interaction between metals in mixtures in plant uptake and toxicity. This information will be critical in the application of these tools to risk assessment of metals mixtures in soil.

WITHDRAWN

624 Improving the Approach for the Ecological Assessment of UVCB Substances

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Ecological risk assessment of complex multi-component substances (e.g. UVCBs - Unknown or Variable Composition, Complex Reaction Products or Biological Materials) pose a challenge to regulators due to these substances' variable and often unknown composition with regard to both identity and proportion of chemical components present in the UVCB substance being evaluated. There are additional challenges in consistently assessing these substances considering the differing levels of complexity and availability and quality of data associated with each UVCB. As part of the next phase of the Chemicals Management Plan (CMP), ECCC is continuing to develop and refine more reliable and consistent methods for the ecological risk assessment of UVCBs under the CMP. In principle, the approach taken for assessing UVCBs is the same as for discrete chemicals where multiple lines of evidence are considered in a weight of evidence approach when assessing a substance's potential to cause harm under the *Canadian Environmental Protection Act*, 1999. In order to evaluate the fate, exposure and effects of a UVCB, experimental data for the UVCB substance as a whole should ideally be evaluated (e.g., whole-substance toxicity tests) in conjunction with data available on individual components and any chemical characterization information.Ultimately, the assessment approach taken will depend greatly on both the complexity of the UVCB and the amount and quality of data available. An approach which takes these factors into account is being considered for the ecological assessment of non-petroleum organic UVCBs. This approach relies on the derivation and use of representative components for assessment and requires establishing sub-classes of known or possible components, followed by selection of a sub-set of protective representative structures to help establish lines of evidence in the assessment.

625 Single and mixture toxicity of TiO2, organic UV-filters and parabens

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In the last few years, concern about the environmental fate and behavior of synthetic chemicals used extensively [1] in beauty and hygiene products has risen. Beside this, there is few information about the toxicity of the engineered nanoparticles (NPs) nowadays frequently used in these products. Water bodies act as the sink for disposal of all these toxicants, which ultimately leads to human and aquatic life exposure [2]. These compounds are released routinely in high amounts in the environment and have low degradability, and hence, they have been considered pseudo-persistent. Moreover, most of them are bioactive, and accumulate in sludge and sediments [3], and bioaccumulate in biota [4] as well as in humans [5]. To address this issue, we conducted toxicity assays to determine the EC_{50} values of three parabens and two UV-Fs using standardized toxicity tests on two aquatic species *Daphnia magna* and *Phaeodactylum tricornutum*. Parabens' 48 h median EC_{50} ranged from 32 to 1.5 mgl^{-1} on *D. magna*, and for TiO_2 the resultant value of EC50 was c.a. 3. mgl^{-1} .For the bioassay with the marine algae, TiO_2 and BzP were studied. For the inorganic sunscreen 72 h EC50 value was 2.27 mgl^{-1} and for BzP 10.61 mgl^{-1}. In this work we also studied the joint toxicity on Daphnia magna for selected binary mixtures of UV-Fs, i.e. TiO_2-BP3, TiO_2-BzP and BP_3-BzP. Synergic action was observed on the endpoint of 48 h mixtures TiO_2-BP3 and TiO_2-BzP but an antagonist behavior in the mixture BP3-BzP. This suggests that TiO_2 increase the toxicity when joint with another compound. Acknowledgements MINECO (SOLAR ProjectRef. 2015801004) and the Generalitat de Catalunya (Consolidated Research Group: Water and Soil Quality Unit 2014-SGR-418). References [1] Díaz-Cruz MS, García-Galán MJ, Barceló D., J Chromatography A 2008, 1193, 50–59. [2] Adam SM, Greeley MS Jr. Establishing possible links between aquatic ecosystem health and human health: an integrated approach. London: Chapman & Hall; 1996. Pp. 91-102. [3] Gago-Ferrero, P., Diaz-Cruz, M.S., Barcelo, D., Chemosphere 2011, 84, 1158–1165. [4] Daughton, C. G, *Environ. Impact Assess. Review,* 2004, 24, 711-732. [5] Valle-Sistac J., Molins-Delgado D., Díaz M., Ibáñez L., Barceló D., Díaz-Cruz M.S., Environ Int. 2016, 88, 243–24.

626 Analysis of Water Accommodated Fractions (WAFs) for Aquatic Toxicity Testing of UVCB substances

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Aquatic toxicity testing required for hazard assessment of chemicals requires consistent and measurable exposures to establish dose-response relationships. Testing of mono-constituent substances, particularly if they are relatively stable and miscible in water, is generally straightforward. However, aquatic toxicity testing of substances of Unknown or Variable composition, Complex reaction products or Biological materials (UVCBs) presents considerable challenges. UVCB components may have different physicochemical characteristics, such as solubility or volatility, and this complicates risk assessment of the UVCB substance through both modelling and testing approaches. Aquatic toxicity remains a critical parameter for chemical risk assessment, but dosing of a UVCB via aquatic media is not straightforward. One standard approach developed in collaboration with Shell scientists was the Water Accommodated Fraction (WAF), in which the test substance is gently stirred in the aquatic test media until equilibration. After a settling period, the test solution is drawn from the base of the vessel to prevent contamination from undissolved material. This method was endorsed for aquatic toxicity testing of petroleum products by CONCAWE (the European industry association for oil companies)[1], and it is an accepted methodology for testing these products (OECD, IMO, ECHA, UN GHS). During aquatic toxicity testing, the concentration of the test substance in the media should be analytically quantified to provide accurate dose response, verify that undissolved matter was not introduced, and confirm consistent exposure concentrations. The analytical quantification of UVCBs in aqueous media is challenging due to constraints in available analytical techniques and the multi-constituent nature. This poster will present a case study of WAF preparation and analysis, using results generated at two independent laboratories. This work illustrates some of the challenges associated with the WAF preparation plus the interpretation of the data generated. [1] CONCAWE (1993). Report 92/56: ecotoxicological testing of petroleum products.

627 Prospective aquatic risk assessment for mixed land use catchments: A tool to combine multi-source chemical emissions over time

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In 2015, a SETAC Pellston® workshop was held to help inform decision making around aquatic mixture risk assessments of chemicals using exposure scenarios for agricultural, domestic, and urban scenarios. Prospective emissions of 37 chemicals were estimated and combined into daily mixture profiles over a 10-year period. The mixture risk assessment looked at daily individual substance risk quotients (RQs) and multiple substance ∑RQ (assuming concentration addition), along with implementation of the Maximum Cumulative Ratio (MCR) approach. Risk was examined at the bottom of a hypothetical catchment containing a changeable configuration of sub-catchments defined by three land use types (agricultural, city [domestic + urban], natural). An underlying spreadsheet-based model was developed to integrate daily loadings of individual chemicals from each sub-catchment, combined with a simplified hydrologic model, to produce a time series of mixture profiles at the catchment outlet. Catchment configuration is changed by varying the placement, type and number of sub-catchments in the system. Model results show a high spatio-temporal variability of individual chemical concentrations and their mixtures based on catchment configuration. Even constant emissions of household chemicals showed variability in concentration related to river flow driven by rain events. The outcome of the overall Pellston study demonstrated that a scenario-based approach can be used to determine whether mixtures of chemicals pose risks over and above any identified using existing approaches for single chemicals, how often and to what magnitude, and ultimately which mixtures (and dominant chemicals) cause greatest concern. In this talk focusing on the underlying catchment model, mixture risk results for different catchment configurations will be presented.

628 Report on the RIFM/ECETOC Workshop: Developing a strategy to improve the environmental risk assessment of difficult to test multi-component substances

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An international workshop was held on 2 – 4 November 2016 prior to the SETTAC World Congress in Orlando FL to address challenges in risk assessing complex mixtures of substances (e.g., multi-constituent substances (MCS), unknown variable composition and biological substances (UVCBs)). International regulatory frameworks (specifically REACH, Canada's DSL Categorization and Chemicals Management Plan assessments, and USEPA's PMN process) have highlighted the complexities of registering, characterizing fate and exposure, and risk assessing complex chemical mixtures whether from manufacturing environments or plant derived materials. Several industrial sectors (e.g., petrochemicals, personal care) have developed frameworks and methodologies for characterization and analysis of these complex substances. This Workshop was designed to identify best practices and key research needs to support environmental risk assessment. An update on post-workshop activities will be presented. The views of the authors of this presentation are those of the authors and do not represent the views of their respective organizations.

Wildlife and Terrestrial Toxicology

629 Exposure to Carbon Nanotubes Elicited Phenotypic and Physiological Stress Responses in Tomato Plants

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Carbon nanotubes (CNTs) are currently used in a wide-range of commercial and agricultural applications. The increasing use of CNTs in fertilizer, pesticide, and herbicide delivery systems could have direct, unintended consequences on soil health, the microbiome, and managed and native ecosystems. Limited studies have examined the impact of CNTs on abiotic plant stress response. In addition, very few studies have evaluated the potential consequences of plant CNT uptake with respect to plant health and subsequent potential human dietary exposure to CNTs. This study observed phenotypic and physiological responses of tomato plants (*Solanum lycopsersicum, cv Ailsa Craig*) exposed to varying forms of CNTs. Tomato plants were exposed through spiked soil (1 mg/kg) to functionalized single-walled carbon nanotubes (SWCNT-OH), non-functionalized (SWCNT-NF), non-functionalized SWCNTs suspended in surfactants (SWCNT-SDBS), functionalized multi-walled carbon nanotubes (MWCNT-COOH), non-functionalized MWCNTs (MWCNT-NF), and non-functionalized MWCNTs suspended in surfactants (SWCNT-SDBS). Differences in plant height, chlorophyll content, flowering time, and fruiting time were observed. MWCNT-COOH exposed plants were found to be significantly shorter and produce less chlorophyll during the first six weeks of growth, when compared to the controls ($P< 0.05$). MWCNT-NF exposed plants produced fruit significantly sooner than controls ($P< 0.05$). Plant stress was evaluated by observing changes in citrulline production (known to increase in plants under abiotic stress) and photosynthetic rate. Preliminary studies found a 10-fold increase in citrulline exposed to CNTs. Results of past studies have varied between plant species and type of CNT. However, whether there is variability in stress response or impact of climate in different species exposed to CNTs has yet to be determined. In an ongoing study, a subset of tomato plants were treated with MWCNT-NF and MWCNT-COOH at 1mg/kg and subjected to water deficit for 14 days. Photosynthetic response will be measured before, during, and after the water deficit. Differences in stress response will be reported. Based on these studies more research is needed on the effects that CNTs have on different crop species under different environmental conditions.

630 The Fate and Effects of Nano Copper Oxide to Earthworms

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Metallic nanomaterials are increasingly used in commercial and manufacturing applications world-wide. Concern over the fate of these materials in the environment has led to efforts to improve our understanding of fate and effects, and how they differ from metal ionic counterparts. Copper (Cu) nanomaterials are used for a wide variety of applications due to its conductive and anti-fungal properties. These nano Cu materials are expected to be released to the environment ending up in the soil compartment either directly through the leaching of nano Cu treated materials (e.g. treated lumber) or, indirectly through the application of contaminated biosolids to agricultural soils. The effects of nano copper oxide (nCuO) on earthworms (*Eisenia andrei*) were evaluated through reproduction toxicity and bioaccumulation tests. A field collected agricultural soil was spiked either directly or through biosolid amendment with 40nm nCuO or, with $CuSO_4$. The fate of the nanomaterial was examined through measurements of test soils for: total Cu (using ICP-MS), Cu^{2+} ionic activity (using an ion selective electrode) and particulate Cu (using ICP-MS in single-particle mode, and TEM). Sub-samples of soil were analyzed throughout the duration of testing (up to 56 days) to investigate dissolution to Cu^{2+}, the formation of aggregates or transformation to other Cu complexes. By understanding the fate in the soils, we can better understand what test organisms are exposed to and, hence the most likely cause for any observed effects.

631 Comparative Study of Two Different Exposure Method of Carbendazim to Earthworm, Eisenia fetida for Lethal, Sub-lethal and Reproductive Effect

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Carbendazim is a common active ingredient in benzimidazole fungicides and is a metabolite of benomyl. It is used to control plant diseases on arable crops, fruit and vegetables. Earthworm toxicity tests are useful tools for terrestrial risk assessment. In this study, the toxicity of carbendazim with two different exposure method was evaluated on different lethal, sub-lethal and reproductive endpoints of earthworm *Eisenia fetida*. In the first method, carbendazim was dissolved in acetone and mixed with artificial soil. After complete evaporation of solvent, earthworms were exposed at different concentrations. In the second method, carbendazim was mixed with 10 g of industrial sand and this treated sand was added to the artificial soil. After proper mixing earthworms were exposed at different concentrations. The method was followed as per OECD 222. The evaluated endpoints included: behaviour symptoms, mortality, body weight, cocoon production and juvenile production (reproductive output). In the first method, the 28 day LC_{50} of carbendazim was determined as 8.26 mg/kg artificial soil. The EC_{50} calculated for reproduction was determined as 1.32 mg/kg artificial soil. The no observed effect concentration (NOEC) on reproduction was 0.7 mg/kg artificial soil and the lowest observed effect concentration (LOEC) on reproduction was 1.3 mg/kg artificial soil. In the second method, the 28 day LC_{50} of carbendazim was determined as 2.81 mg/kg artificial soil. The EC_{50} calculated for reproduction was determined as 0.68 mg/kg artificial soil. The no observed effect concentration (NOEC) on reproduction was 0.4 mg/kg artificial soil and the lowest observed effect concentration (LOEC) on reproduction was 0.8 mg/kg artificial soil. Carbendazim was found highly toxic with adverse behavioural symptoms to *E. fetida*, significantly reducing earthworm weight, production of cocoon and juvenile. The mixing of carbendazim with sand is more effective and sensitive method than mixing with acetone.

632 Layer-Performance and Egg Quality of Domestic Fowl Exposed to Dichlorvos

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Use of pesticides in agriculture and domestic purposes may have adverse effects on humans and non-target animals. Dichlorvos is widely used as an insecticide to control household pests, in public health, protecting stored product from insects and control of parasites in livestock. This study was carried out to evaluate the reproductive performance of laying hens exposed to dichlorvos. Seven weeks old pullets with an average weight of 557.5 ± 9.5 g were divided into four groups and fed *ad libitum* with commercial poultry feeds contaminated with 0.01, 0.02 and 0.04% dichlorvos (w/v). The control group had no pesticide added to their feed.

The pesticide exposure was continued until nine weeks after laying of eggs. There was a significant reduction ($p< 0.05$) in feed intake between the control group and those exposed to dichlorvos. Egg laying was delayed in the hens exposed to pesticides by as much as eighteen weeks. The ages of the hens at first egg lay were 18 weeks for the control, 23 weeks for hens fed on 0.01 and 0.02% contaminated diet and 36 weeks for those fed on 0.04% contaminated diet. The average daily egg production was reduced from 5 eggs in the control group to 1 egg in 0.04% contaminated group. The protein contents of the egg (yolk and albumin) and cholesterol level of the egg yolk were lower in birds exposed to pesticide. There was no significant difference in the weight of eggs between the control and those exposed to pesticide. Results of this study suggest that exposure of poultry birds to dichlorvos could affect their reproductive success.

633 Mercury concentrations in Louisiana marsh birds and small mammals on opposite sides of the Mississippi River plume

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The current understanding of the origin and transport of mercury (Hg) in the Gulf of Mexico is lacking. This is in contrast with the importance of Gulf fisheries to overall U.S. national fish consumption, particularly given evidence of higher Hg concentrations in Gulf fishes compared to the U.S. Atlantic coast. The Mississippi River is an important contributor to Hg levels in the Gulf, through direct input and via boosting of biological productivity and Hg methylation. Yet, Hg biota concentrations in areas that differ in their exposure to the low-salinity waters of the Mississippi River plume (MRP) remain to be characterized. Here, we quantified total Hg levels (THg) in blood samples collected during 2014-2015 from 77 marsh rice rats (*Oryzomys palustris*) and 184 seaside sparrows (*Ammodramus maritimus*) in southeastern Louisiana, West and East of the MRP (in Barataria Bay and Breton Sound, respectively). In both species, THg concentrations were higher East than West of the MRP [marsh rice rats, East: 0.058 ± 0.006 µg/g ww (mean ± SE), n = 18; West: 0.022 ± 0.002 µg/g ww, n = 59; seaside sparrows, East: 0.188 ± 0.013 µg/g ww, n = 28; West: 0.099 $\pm$ 0.003 µg/g ww, n = 156]. In addition, THg concentrations were higher in adult seaside sparrows compared to juvenile seaside sparrows, potentially indicating lifetime accumulation of Hg. The present results indicate higher Hg biota concentrations in coastal marshes more likely exposed to freshwater input from the MRP. These findings have important implications for modeling Hg input from future controlled river diversions, and for Hg delivery from the Mississippi estuary to marine waters.

634 Can Avian Reproductive Outcomes Estimated With MCnest Be Made More Robust Using Stochastic Parameterizations?

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The Markov chain nest productivity model, or MCnest, is a set of algorithms for integrating the results of avian toxicity tests with reproductive life-history data to project the relative magnitude of chemical effects on avian reproduction. The mathematical foundation of MCnest is in the analysis of Markov chains, which provides a flexible template for modeling the variation in avian breeding cycles among species. MCnest quantitatively estimates the relative change in the number of successfully fledged broods per female per year of avian species exposed to a specific pesticide application scenario. The relative change in the number of successful broods is estimated by comparing model results based on a defined pesticide application scenario with a no-pesticide scenario. MCnest requires data from avian toxicity tests, avian species life-history profiles and a pesticide-use scenario that defines the timing and temporal pattern of exposures. In addition, MCnest uses algorithms from the USEPA Office of Pesticide Programs Terrestrial Residue EXposure model or T-REX to translate the application rate (expressed as pounds AI/acre) into doses (expressed as mg/kg body wt./day) for both adult and juveniles taking into account the species typical diet. Under ideal circumstances, McNest estimates would be reflected in similar real world occurrences in terms of species reproductive outcomes following pesticide exposures. Validation of model estimated outcomes versus empirical data can however be difficult, particularly in light of the use of deterministic input of sensitive parameters. The goal of the current analysis is to employ a stochastic approach to model parameterization in terms of toxicity endpoints, life history variability, exposure timing and magnitude in order to present a robust profile of avian reproductive outcomes as a function of variability in selected sensitive input parameters. Finally, modeling outcomes accounting for the variability in sensitive input parameters are to be compared with a set of empirical data in order to ascertain the success of the stochastic approach.

635 Bald Eagles (Haliaeetus leucocephalus) as a Bioindicator of Contaminant Trends in Aquatic Ecosystems

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Bald eagles (*Haliaeetus leucocephalus*) are piscivorous tertiary predators that have been used as indicators of chemical contamination in aquatic habitats. The Michigan Bald Eagle Biomonitoring Program has monitored organochlorine pesticides (OCs) and polychlorinated biphenyls (PCBs) from 1999 through the present day in bald eagle nestling plasma, which is a direct reflection of their immediate prey base within parental foraging distance. Plasma samples are digested with concentrated urea and extracted using a solid phase extraction method using a Waters hydrophilic-lipophilic-balanced HLBâ cartridge to allow for maximum retention of contaminants, and reconstituted in hexane. 42 contaminants are measured via gas chromatography at the University of Maryland Joint Analytical Services Laboratory. Spatial and temporal analyses from 1999 through 2013 were conducted by sampling ~90 samples throughout 20% of the state every year, with the entire state being fully sampled every five years. A newer analysis allows for 100% sampling of the entire state every year with only ~60 samples, and has been shown to produce the same trends in data spatially and temporally. The data in this update from 1999 through 2013 were analyzed with the five-year analysis while the 2014-2017 data were analyzed using the annual analysis. Overall spatial trends indicate that areas within immediate proximity of the Great Lakes have higher levels of these contaminants than inland regions. Temporal trends indicate that contaminant levels are falling but that there may be new sources of introduction into the environment.

636 Estimating Lead Risks to Songbirds: Dietary Doses Versus Blood Bioaccumulation Estimates

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When dietary toxicity reference values (TRVs) based on the most lead-sensitive bird species – Japanese quail (*Coturnix japonica*) – are applied to exposure estimates for songbirds such as the American robin (*Turdus migratorius*), adverse reproductive effects can be predicted at soil lead concentrations that are within the range of naturally occurring background concentrations. Some researchers have posited that Japanese quail reproduction may be atypically sensitive to lead. Further possible explanations include (1) a distortion in interspecies extrapolation due to the very different body sizes of quail and robins, and (2) differences in bioaccessibility between lead in robins' diet and lead administered in quail toxicity tests. Exposure assessment based on blood lead concentrations provides a line of evidence that avoids uncertainties associated with dietary dose extrapolation. Two methods for estimating lead-related risk to birds are compared using data from published literature: (1) dietary doses estimated using soil-to-invertebrate bioaccumulation data and species-specific soil and food ingestion rates compared to dose-response relationships for Japanese quail and other test species, and (2) blood lead concentrations estimated using soil-to-blood bioaccumulation data compared to blood-based TRVs. For the dose-response assessment, a total of nine lead toxicity studies were reviewed. Test species included American kestrels, chickens, Japanese quail, rock doves, and ring-necked turtle doves. For the blood-based assessment, paired soil and blood lead concentrations were compiled for American robins and European blackbirds (*Turdus merula*) from four sites. For a given soil lead concentration, the blood-based approach tends to yield lower risk estimates than the dose-based approach.

Emerging Environmental Chemistry – Trends, Transformations and Fate of Organic Environmental Contaminants – Part 2

637 Non-target analysis in reclaimed water using online SPE coupled to HPLC-ESI/HRMS

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The advancements in the field of analytical chemistry, and especially mass spectrometry, have been redefining the field of contaminant detection. Non-target analysis requires no pre-selection of compounds and have become very popular in the last years for the determination of new and emerging contaminants, or transformation products. Reclaimed water as the end product of wastewater reclamation are mainly used in landscape and agricultural irrigation, ground water recharge and recreational purposes. However, besides all the economic benefits of water reuse, this type of water still contains high levels of contaminants which are not properly eliminated by the treatment process. In this study, the detection of unknown compounds in reclaimed water was carried out by a Q-Exactive Orbitrap mass spectrometer and subsequent data processing and identification of unknown compounds was performed using the Compound Discover 2.1 software. A fast and straight forward online solid phase extraction (SPE) followed by high performance liquid chromatography-high resolution mass spectrometric (HPLC-HRMS) analysis was developed using a Hypersil Gold C18 column for separation and acetonitrile and 0.1% formic acid as the mobile phase. Analysis were performed in 13 minutes and full scan of 140,000 resolution in both positive and negative modes. Over 1000 compounds were detected and identified in negative and positive modes, which included herbicides, pesticides, pharmaceuticals and personal care products, among others.

638 Metabolism of Pharmaceuticals in Higher Plants

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Reclaimed water is a historically underutilized resource, but population growth and global climate change have placed increased pressure on current water resources. Reclaimed water has become a useful tool to meet the water needs of citizens, industries and agriculture. The use of reclaimed water, however, comes with the potential risks of environmental and food contamination by pharmaceuticals. The levels of pharmaceuticals in plants will depend both on translocation and metabolism in plant tissues. Currently most studies have only attempted to determine the concentration of pharmaceuticals' parent compounds taken up by plants. As such very little is known about the formation of pharmaceutical and personal care product (PPCP) metabolites in plants. Research conducted in this laboratory has shown that plants are capable of metabolizing pharmaceuticals with phase I modification, followed by phase II conjugation and phase III translocation and sequestration. We have analyzed the metabolism of the pharmaceuticals carbamazepine, diclofenac, sulfamethoxazole and diazepam in plant cell cultures and whole plants grown hydroponically. These compounds were selected because of their common occurance in reclaimed water. We employed time-of-flight (TOF) and triple quadrupole (QqQ) mass spectrometers, ^{14}C tracing, and enzyme assays to determine the products of phase I-III metabolism. Phase I metabolism consisted of products created from oxidation and hydroxylation reactions while phase II metabolism saw products formed from rapid conjugation with glucose, glutathione, and amino acids, as well as acetylation reactions. Both cell cultures and whole plants saw extensive phase III metabolism with > 50% of each compound becoming non-extractable or bound residues by the end of the incubations. The extensive formation of bound residues and conjugates highlights the need to considered metabolism when assessing risk of pharmaceuticals entering the environment. Our research shows that metabolic processes may be masking the actual concentration of PPCP compounds in the environment and thus the risks to human and environmental health.

639 Suspect Screening of Emerging Contaminants in Onondaga Lake and Its Tributaries Using High-Resolution Mass Spectrometry

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Onondaga Lake, an urban lake located in Syracuse, has a long history of industrial pollution (e.g., mercury) and is a major source of water to Lake Ontario. The lake receives a high volumetric input of treated sewage (representing ~20% of the total lake inflow) from a large municipal wastewater treatment plant (WWTP) serving the Syracuse metropolitan area. However, the occurrence, fate, and risk of emerging contaminants (ECs) in the Onondaga Lake system remains largely unknown. The main objectives of our study are to apply a suspect screening approach based upon Orbitrap mass spectrometry to establish the occurrence patterns of ECs in this lake system and to identify priority compounds to inform future monitoring efforts. Grab samples were collected from Onondaga Lake, its six major tributaries, the WWTP outlet as well as Otisco Lake located upstream of Syracuse. Samples were processed by solid-phase extraction and analyzed on an LTQ Orbitrap XL high-resolution mass spectrometer with positive electrospray ionization. For suspect screening, a database containing 490 compounds was compiled by combining analytes targeted in the USGS Methods 5-B10 and 5-B11 and ECs previously detected in New York streams and lakes. 130 ECs were identified as suspect hits (detected at least once). The average number of suspect hits followed the order of WWTP effluent (99) > Onondaga Lake (60) > tributaries ≈ Otisco Lake (~35). 52 out of 60 ECs detected in Onondaga Lake overlapped with those found in the WWTP effluent, highlighting the WWTP as an important point source of ECs. 22 ECs were confirmed in 9 water samples by the standard compounds (confirmed at least once). Ongoing work in our laboratory is focused on the confirmation and retrospective quantification of suspects, and the photochemical degradation of ECs. These results could provide a knowledge base from which subsequent studies can be designed to more comprehensively characterize the fate of ECs in the Onondaga Lake system.

640 Subsurface filtration technology to attenuate pharmaceuticals, nutrients, antibiotic resistance and toxicity for wastewaters in municipal wastewater

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The occurrence of wastewater contaminants from lagoon to release at a new treatment facility in Dunnottar, Manitoba was characterized and compared to a pilot-scale system previously installed on the same site. Wastewater treatment was performed by a two-lagoon system with subsequent subsurface filtration and ultraviolet treatment. Grab samples were collected during the Summer of 2015 and 2016 to measure pharmaceuticals, antibiotic resistant gene (ARG)-bearing organisms and toxicity along the wastewater path, and Polar Organic Integrative Chemical Samplers (POCIS) were deployed at the input, output and post-UV treatment on the filtration system to passively collect pharmaceuticals. Nutrient concentrations were grab-sampled and measured at the input and output of the filter in 2015. Attenuation was observed for nutrients at the output of the subsurface filter (31% ammonia declines; 61% total phosphorus declines). Only five pharmaceuticals were consistently detected throughout the treatment system: atenolol, sulfamethoxazole, metoprolol, propranolol and carbamazepine. For these five, most removal occurred in the primary lagoon (up to ~99% atenolol, 73% sulfamethoxazole, 86% metoprolol, ~99% propranolol, 56% carbamazepine), with no apparent attenuation being observed within the filter, which is consistent with what was found for these contaminants at the pilot-scale filter on our previous study. Pharmaceutical concentrations at the time of the study did not represent a hazard for aquatic species. Overall, the full-scale lagoon-filter system was able to attenuate all wastewater contaminants and toxicity to a similar or superior degree when compared to the pilot-scale filter with non-significant effects due to seasonality. We conclude that the full-scale lagoon-subsurface treatment filtration system can provide a low-cost and low-maintenance means to reduce commonly found contaminants in municipal wastewaters.

641 Efficiency of tertiary treatment in wastewater treatment plants for mineralization or transformation of antidepressants and their impact on zebrafish

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Prescriptions of antidepressants to humans has escalated over the years due to increasing population and demand. After disposal or excretion of antidepressants they travel to the wastewater treatment plants (WWTPs). The WWTPs have multiple treatment stages and transformation or mineralization of these compounds has widely been attributed to the tertiary treatment stage. The release of antidepressants into the aquatic environment is of grave concern as it has been correlated with negative health effects in fish, including: lethargy, change in eating habits, and decrease in reproduction. Moreover, the formation of transformation products from the system processes can result in more potent forms of the drug being released in the effluent. There are multiple processes available for the tertiary treatment stage in WWTPs including ozone, biofiltration, and granular activated carbon. Though these three different options for tertiary treatment processes are all used in modern WWTPs, it is unknown which is the most effective for organic pollutant mineralization. In this study, the efficiency of antidepressant removal is examined by analyzing 24-hour composite samples of the influent and effluent of these tertiary treatment processes. Samples were analyzed using a targeted LC-MS/MS method for a suite of seventeen antidepressants including: selective serotonin reuptake inhibitors, serotonin-norepinephrine reuptake inhibitors, and norepinephrine–dopamine reuptake inhibitors.Next, transformation products of analytes were analyzed using high resolution mass spectrometry in order to determine if there is a statistical difference between the influent and effluent from each tertiary treatment process. Furthermore, exposure studies of zebrafish embryo to antidepressants are performed to evaluate how their growth and development compares to a control. Preliminary data shows differences between the influent and effluent antidepressants levels.

642 Micropollutants in Groundwater and Soil Downgradient from On-Site Wastewater Discharges

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Several methods of on-site wastewater treatment systems are currently used throughout the United States. However, wastewater discharges from on-site treatment systems can contain micropollutants such as pharmaceuticals, personal care products, and other organic wastewater chemicals, potentially affecting soil and shallow groundwater. We evaluated the presence of micropollutants in shallow groundwater near wastewater land application sites located in vulnerable aquifer settings in Minnesota. Three types of sites were targeted: (1) large subsurface treatment systems (LSTS), (2) rapid infiltration basins (RIB), and (3) an agricultural field that irrigates with treated wastewater. Thirty-four micropollutants were detected at LSTS and RIB sites, 18 of which were pharmaceuticals. Maximum concentrations ranged from 1.1 (glyburide) to 960 (sulfamethoxazole) nanograms per liter. At the agricultural field, shallow groundwater, as well as soil, was sampled for micropollutants. Three micropollutants were detected in groundwater, 32 were detected in soil samples. Micropollutants detected in soil included fragrances, alkylphenols, flame retardants, hormones, and antibiotics. Two antibiotics, ciprofloxacin and ofloxacin, were detected at concentrations above the limit of quantification (>1 milligram per kilogram). Results indicate that these land application methods potentially contribute to micropollutant loading of surface waters via groundwater discharge.

643 New Mass Spectrometry Techniques for Measurement of Persistent Organic Pollutants

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Recent development of new mass spectrometry technology and instrumentation has increased the amount and quality of analytical information that can be obtained from samples. In particular, dramatic increases in mass resolution have made possible unequivocal identification of contaminants even in complex mixtures and matrices. In the area of POPs analyses of PCDD/Fs and PCBs are of concern due to small concentrations that need to be quantified and the presence of a wide range of other chlorinated chemicals that might potentially interfere. The recent release of a GC/OrbiTrap system brings levels of mass resolution not previously available for analysis of POPs by GC chromatography coupled with ultra-high resolution mass spectrometry (GC-UHRMS). Here we report use of GC-UHRMS for identification and quantification of PCDD/Fs and PCBs. The methods developed are based on standard US-EPA methods (Methods 1613 and 1668) but are enhanced by use of new capabilities provided by image current detection and high mass resolution (> 100,000 FWHM). Robustness of the PCDD/F analyses were demonstrated by excellent calibration characteristics and ability to detect all 2,3,7,8-substituted congeners even in an extract of used motor oil. Reanalysis of fish tissues previously analyzed by a magnetic sector instrument demonstrate accuracy of identifications and quantifications and provide validation of the method. Analyses were also conducted to determine the potential for a ‘mutiplex‘ analysis of various POPs where the resolving power (>120,000 FWHM) of the MS system was able to eliminate potential interferences from a variety of ‘non-target’ organochlorines. These multiplexed analyses significantly reduce the time and cost of sample extract preparation and clean-up. In addition, the relatively simple auto-tuning and mass calibration algorithms available for the advanced mass spectrometry systems greatly simplify these otherwise challenging analyses.

644 Quantitative Structure-Reactivity Relationships of Hydroxyl Radical Rate Constants for Linear and Cyclic Volatile Methylsiloxanes

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The accurate understanding of the fate of volatile methylsiloxanes (VMS) in air is crucial in determining their persistence and concentrations in the environment. Although oxidation by atmospheric hydroxyl radicals (•OH) is considered as a major degradation mechanism for airborne VMS, the existing bimolecular rate constants with •OH measured and modeled for any given VMS compound varied greatly, depending on the approaches used to generate the data. The objectives of this study were to measure •OH reaction rate constants for four cyclic and four linear VMS based on a relative rate method using a newly designed atmospheric chamber and to establish structure-reactivity relationships for the kinetics. In the past, the reaction rate constants for VMS were generally recognized to increase with the number of the methyl groups per molecule, the only differential factor in the existing models. However, the new measurements indicated that molecular structure should be additionally considered in the prediction of the reaction rates. The measured data showed that •OH reaction constants normalized to the number of methyl group (N_T) range from 1.2 to 1.5×10^{-13} cm³ molecule^{-1} s^{-1} N_T^{-1} for four cyclic methylsiloxanes (D3, D4, D5, and D6) and from 2.6 to 3.4×10^{-13} cm³ molecule^{-1} s^{-1} N_T^{-1} for four linear methylsiloxanes (L2, L3, L4, and L5) at 25 °C. Better empirical models were developed by simple and multiple linear regressions of the measured values from this study and the literature. A high correlation existed for the reaction rates with the number of methyl group attached at two distinct siloxane structures (i.e., linears and cyclics). Even better correlations were obtained with one or two molecular descriptors that are directly related to the size of VMS, which, in turn, not only depend on the number of methyl groups, but the linear/cyclic structures as well for permethylsiloxanes.

Ecotoxicology and Environmental Health in the Developing World

645 Towards Sustainable Environmental Quality: Ecotoxicology and Environmental Health Research Needs in Developing Countries

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Global megatrends such as demographic transitions, urbanization and the food-energy-water nexus continue to transform international relations, while stressing critical resources and affecting public health and the environment. Many of the United Nations sustainable development goals (SDGs: www.globalgoals.org) depend on implementation of sound environmental management decisions, based on credible scientific evidence. The Global Horizon Scanning Project aims to identify geographically specific research needs to address stressor impacts on environmental quality, and thus to support achieving the SDGs. Priority research questions were solicited from Society of Environmental Toxicology and Chemistry (SETAC) members, members of the American Chemical Society's Environmental Chemistry and Agrochemicals Division, and other environmental professionals within the five SETAC geographical units (GU). Input was synthesized by expert teams of academic, industry and government representatives to form lists of key research questions that, if answered, would substantially advance our understanding of how a range of environmental stressors (chemical, physical, biological) impact environmental quality in different geographic regions. Workshops were held in conjunction with SETAC Africa (in Langebaan, South Africa), SETAC Europe (in Barcelona, Spain), SETAC Latin America (in Buenos Aires, Argentina), SETAC North America (in Salt Lake City, USA), and SETAC Asia-Pacific (in Nelson, New Zealand, for Oceania; in Singapore for Asia). Each workshop was chaired by SETAC members representing academic, business and government sectors. Questions received were scientific in orientation, and covered diverse aspects of fields related to SETAC's mission to advance sustainable environmental quality. This presentation provides a synthesis of major ecotoxicology and environmental health research themes emerging within and across SETAC Geographic Units with a particular focus on developing countries, where coupled ecological and human health needs are readily apparent. It is anticipated that outputs from this effort will help increase the quality and relevance of environmental research, decrease scientific uncertainty in assessing and managing ecological and health risks, and increase the credibility of technical and policy responses to global environmental stressors.

646 Occurrence and potential ecotoxicological impacts of organochlorine pesticides in the riverine environments of Syr Darya, Ganga and Brahmaputra Rivers

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The availability of freshwater for multiple purposes is presenting an increasingly serious challenge for the developing world due to the rapidly growing economy, increasing industrialization and agricultural sectors. The Syr Darya in Kazakhstan and Central Asia, and Ganga-Brahmaputra from Southeast Asia are three transboundary rivers forming an integral part of the meltwater resources of these regions. Organochlorine pesticides (OCPs) are legacy persistent organic pollutants (POPs), used extensively by these Asian countries over the past half century. OCPs are generally hydrophobic and lipophilic compounds with endocrine disrupting properties. Systematic monitoring of pesticide residues and degradation products is generally lacking in Syr Darya and Ganga-Brahmaputra River Basins, and it will be critical to assess where freshwater quality is inadequate and under threat. In this study, we are reporting the seasonal levels of organochlorine pesticide residues in the riverine environment. Associated eco-toxicological risk was assessed using the Hazard Quotient formula given by USEPA. River water and sediment samples were collected from Syr Darya (n=5), lower stretch of Ganges (n=22) and Brahmaputra (n=18). Grab samples of water and sediment were extracted and analyzed using gas chromatography mass spectrometry. Among OCPs measured, γ-HCH showed maximum detection frequency in all the three rivers. The occurrence of *p,p'*-DDE in some of the riverine sediment samples is likely from past usage of technical DDT. Other OCPs were below the detection limit for Syr Darya. The detection frequency was nearly 90% for the quantified OCPs in Ganga and Brahmaputra with dominance of DDT, HCH and Heptachlor. Abundance of *p,p'*-DDT mostly in lower stretch of Ganga reflects the usage of technical DDT. Lindane (99% γ-HCH) was heavily used in India, China and other southeast Asian countries until it was recently banned. Given the fact that Kazakhstan did not have a history of lindane usage, γ-HCH in Syr Darya may have resulted from atmospheric deposition. While the impact of elevated DDT and lindane concentrations was limited to lower trophic level species, endosulfan showed impact on an edible fish species in specific sites of Ganga and Brahmaputra. Deterioration in freshwater quality due to continued occurrence of modern and legacy pesticide residues may pose a risk for public health, biodiversity and the aquatic ecosystem.

647 Occurrence of Brominated Flame Retardants and Other Persistent Organic Pollutants in Fish, Air, and Sediments in the Philippine Environment

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The occurrence of halogenated persistent organic pollutants in the environment has increased over the years due to industrialization. Halogenated chemicals, especially polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), are of particular interest because human exposure to these compounds has been linked to adverse health effects, including disruption of the endocrine system, neurodevelopmental delays, and carcinogenic effects. The PBDEs are mixed into polymers for use as flame-retardants in products such as electronic circuit boards and cases, textiles, vehicles, synthetic building materials, and carpeting. PBDEs are easily leached from these products and are released into the environment. Human exposure to these compounds is mainly through inhalation of PBDE-containing dust particles, as well as ingestion of contaminated food products such as fish. PBDEs may be released into the Asian environments at unprecedented amounts, and considering that fish is a major part of the Filipino diet, assessment of the levels of PBDEs in the Philippine human population, environment, and food sources is warranted. Through international collaborations with local universities in the Philippines, a developing Asian country, experimental protocols have been established to conduct the sample collection, extraction, and analysis of fish, sediments, and dust particles, specifically PM_{10} [Particulate matter, 10 microns or less] collected within Manila, Philippines. The PM_{10} are very small solid particles in the air that are of particular concern because these particles are easily inhaled and can be readily absorbed into the lungs, introducing contaminants associated with dust particles into the human body. Fish samples that are commonly consumed were purchased from the local grocery stores and the Farmers market for extraction and analysis. Sediment samples were collected in Manila Bay and its tributaries and were analyzed for PCBs, PBDEs, and legacy pesticides including DDT. An overview of these efforts and preliminary results will be presented, and will include challenges and limitations, and potential solutions for conducting research in developing world.

648 Using Passive Sampling to Measure Bioavailable Pesticide Exposure in the Red River Watershed of China and Vietnam

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Current-use pesticides are generally less persistent and more polar/hydrophilic chemicals than their chlorinated predecessors. Additionally, acute pesticide poisoning events are very rare and pesticide application rates are very low, so the predominant environmental concern is long-term chronic exposure at very low concentrations of pesticides. This presents new challenges in estimating environmental exposure and assessing human and ecological risks associated with pesticides in aquatic systems. Several types of passive sampling devices (PSDs) have been developed for monitoring chronic chemical exposure in aquatic systems however their use is largely limited to hydrophobic chemicals. We have developed a more universal non-selective passive sampling device (nsPSD) that has been calibrated in the laboratory for 35 current-use pesticides and 21 organochlorine pesticides with log K_{OW} values ranging from 1.49-8.15. The nsPSD was deployed along with the polar organic chemical integrative sampler (POCIS) and low-density polyethylene (LDPE) sheets in the Red River watershed starting near Dali, China and ending in Hanoi, Vietnam. We found that the time-weighted average (TWA) exposures estimated using our new nsPSD compared well with those obtained using the LDPE for chemicals with log K_{OW} above about 5.0. The agreement was not as good between the nsPSD and the POCIS, with many chemicals detected in the nsPSD not being detected in the POCIS or the POCIS having much lower accumulation than the nsPSD. At selected sites, aquatic invertebrate toxicity testing was also performed and the toxicity results were in good agreement with pyrethroid insecticide concentrations estimated from the nsPSD and the LDPE. Overall, we found pesticide exposure in the Red River was low (0.1-10 ng/L range) and any higher concentrations were largely in more stagnant waters very near the location and time of pesticide application. Our results indicate that the nsPSD provides a very good estimate of TWA pesticide exposure over a much wider range of log K_{OW} than either the LDPE or POCIS and that the nsPSD and the LDPE both provide a good measure of the bioavailable fraction of pyrethroids in water.

649 Assessing Environmental Chemical Pollutant Impacts on Waterborne Pathogen Emergence and Evolution in Haiti

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Our understanding of environmental pollutant sources, fate, and impacts in developing countries is limited despite the recognized association between chemical exposure and adverse health outcomes. A major challenge to identifying components of chemical mixtures in surface and groundwater in developing nations is the lack of commercial information about consumer and industrial chemical use. Given this limited information, efforts in assessing waterborne health risks in nations such as Haiti have primarily focused on pathogens, such as toxigenic *Vibrio cholerae*, (*V. cholera).* Such infectious agents continue to have negative impacts on public health, particularly after adverse environmental events such as frequent rainfall associated with extreme weather (e.g. hurricanes). A surprising recent discovery has been that naturally occurring compounds in water (i.e. phosphorous and chitin) can directly influence the growth and phenotype of toxigenic *V. cholera* suggesting that chemical constituents could directly change the behavior of human pathogens in their environment, perhaps allowing enhanced survival under extreme and nutrient-deficient conditions (termed 'persister' phenotype). Because of the lack of toxicology assessments and new knowledge of the impacts of water quality on *V. cholerae*, we have merged these concepts to assess holistic organic chemical contaminant profiles in environmental waters of Haiti and to determine their influence on *V. cholerae*. This presentation will introduce our approach to addressing such issues, spanning disciplines of toxicology, microbiology, geospatial mapping, and non-targeted chemical contaminant analysis. Our initial studies have focused on defined the geospatial relationship between chemical profiles, toxicogenic *V. cholerae,* water quality and meteorological parameters for 17 sites in Haiti. Chemical fingerprints were generated by non-targeted mass spectrometry and using this approach we were able to identify industrial, pharmaceutical, and natural chemical contaminants in areas where toxigenic *V. cholera*e was detected. Identified chemical contaminants are currently being tested for their ability to alter *V. cholera* growth and genetics in laboratory-based microcosms studies. Overall, these data lay the foundation for success for contemporary approaches to identification of chemicals in surface and ground water sources in the developing world and their potential to influence microbes of public health importance.

650 Development of a Native Sentinel Species, Trichomycterus areolatus, for Biomonitoring of Agricultural Pollutants in Central Chile

J.M. Ali, University of Nebraska Medical Center / Environmental, Agricultural and Occupational Health; A.S. Kolok, University of Nebraska-Omaha / Nebraska Watershed Network / Biology

Considerable economic and technical resources have been devoted to monitoring and assessing the occurrence of agricultural contaminants in North American watersheds. One successful approach to this issue is the use of sentinel organisms for biomonitoring of adverse exposures. Often, the ideal sentinel organism is a native species that allows for a "canary in the coal mine" approach to exposure assessment and biomonitoring. Despite its own intense agricultural productivity, the South American country of Chile has a relatively limited capacity for biomonitoring in regional surface waters. Much of the country of Chile is a arid environment with unique challenegs relative to the discipline of ecotoxicology. Mountain Valley river systems, such as the Choapa, run a relatively short distance from their origins in the Andean Mountains to the Pacific Ocean and are separated by kilometers of desert. In this environment, we have identified a native pencil catfish, *Trichomycterus areolatus*, that may be useful as an environmental sentinel. We have annotated the transcriptome of *T. areolatus* using tissue samples collected from the Choapa River in Coquimbo (Region IV), Chile. Based upon this data, we have identified differences in hepatic gene expression downstream of pollution sources along the Choapa River. Among other site specific changes in hepatic gene expression, we found defeminizing endocrine disruption of females collected downstream of intense agricultural activity. This presentation addresses the challenges of developing biomonitoring tools in a developing scientific setting, like Chile, as well as identifying opportunities to improve ecotoxicology in unique and remote locations.

651 Challenges of setting up a water quality testing lab in the developing world: A case study in Haiti

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A large majority of outreach efforts in resource poor countries are focused on infectious diseases that plague these areas. While these are important issues, unregulated chemical use and discharge, environmental degradation, and inadequate treatment infrastructure may also lead to chemical contamination of aquatic resources used for drinking, cooking, cleaning, and fishing. Yet little work has been done to characterize the presence and potential environmental health risks of aquatic chemical contaminants in resource poor countries because there are significant challenges in advancing water quality testing in developing countries including access to equipment, developing a client base, recovering costs of testing, and navigating existing governmental processes for lab certification. Haiti is located on the island of Hispaniola and is recognized as the poorest country in the western hemisphere. It has been plagued with widespread environmental health issues resulting from poor infrastructure, little regulation, lack of education, and a receding economy. In Haiti, little attention has been paid to chemical contaminants in aquatic systems. However, there is increased interest among locals, non-governmental agencies, missionaries, industry, and foreign assistance agencies in understanding whether water resources are safe. Working with a group of students in a small-business consulting course called "GatorNest", which is taught by the University of Florida's Entrepreneurship and Innovation Center, our team set out to tackle cost analysis and logistical issues of setting up a certified water testing lab in Haiti. An analysis of equipment, supplies, labor, and facility costs was conducted to determine a sustainable cost of testing. Additionally, we explored to process for government certification, which is essential for client buy in. Our preliminary analysis indicates that though a process exists for becoming a certified water testing lab in Haiti, there are currently no certified labs in the country. The results of the cost analysis are forth coming and will be presented. Based on this analysis, we hope to pilot a water quality lab in Haiti that we can eventually transition to a Haitian run business. Lessons learned from our work in Haiti are useful as toxicological assessments in the developing world gain traction in support of improving ecosystem and human health.

Exposure and Effects of Emerging Contaminants on Aquatic Ecosystems

653 Persistent organic pollutants and contaminants of emerging concern in Minnesota fish

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The results from a survey of selected persistent organic pollutants (POPs) and contaminants of emerging concern (CECs) in Minnesota fish will be presented. A total of 45 archived fish homogenates were analyzed for chlorinated paraffins (CPs), polychlorinated naphthalenes (PCNs), hexabromocyclododecane (HBCD), polychlorinated biphenyl (PCBs) congeners, chlorinated dioxins and furans, and perfluoroalkyl substances (PFASs). The purpose of this screening-level study was to determine whether these POPs were present in Minnesota fish, and if so, whether these POPs were present at levels of concern for either human or ecosystem health. Homogenates selected for analysis had been previously collected at three sites in the Twin Cities Metro Area (TCMA), including Lake Johanna (2010) and Mississippi River Pools 2 (2011) and 3 (2012). High concentrations of several POPs were measured in all fish from all sites.

654 Biomarker Responses to Beta Blocker Exposures in Marine Bivalves

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Increased consumption and improper disposal of prescription medication, such as beta (β)-blockers, contribute to their introduction into waterways and pose threats to non-target aquatic organisms. Beta-blockers are widely prescribed for medical treatment of hypertension and arrhythmias. They prevent binding of agonists, such as catecholamines, to β-adrenoceptors. In the absence of agonist induced receptor activation, adenylate cyclase activation and increases in blood pressure are limited. With their widespread use, there has been rising concern about the impacts of β-blockers on coastal ecosystems, especially because wastewater treatment plants are not designed to eliminate these drugs from the discharge. Few studies have characterized the sublethal effects of β-blocker exposures in marine invertebrates. The aim of our research is to evaluate cellular biomarker responses of two commercially important filter-feeding marine bivalves, Eastern oysters (*Crassostrea virginica*) and hard clams (*Mercenaria mercenaria*), upon exposure to two β-blocker drugs, propranolol and metoprolol. Bivalves were obtained from Narragansett Bay (Rhode Island, USA) and acclimated in the laboratory. Following acclimation, gills and digestive gland tissues were harvested and separately exposed to concentrations ranging from 0-1000 ng/l of each drug for 24 hours. Tissues were bathed in 30 parts per thousand filtered seawater, antibiotic mix, nutrient media, and the test drug. Tissue samples were analyzed for biomarker assays including tissue damage (lysosomal membrane destabilization and lipid peroxidation), total antioxidant capacity, and activity of glutathione-s-transferase (GST) – a detoxification enzyme. Elevated tissue damage and changes in GST activities were noted in the exposed tissues at environmentally relevant concentrations. Digestive gland tissues were more responsive to the exposures than gill tissues. Differences in species sensitivities and responses to the exposures were also observed. These studies enhance our understanding of the potential impacts of prescription medication on coastal organisms, and demonstrate that filter feeders such as marine bivalves may serve as good model organisms to examine the effects of water soluble drugs. Evaluation of a suite of biomarkers allows us to better define molecular initiating events and subsequent key events that might be used to develop adverse outcome pathways (AOPs) for unintended environmental exposures to β-blockers.

655 Toxicological effects of three replacement antimicrobials of triclosan and triclocarban to two model organisms the nematode C. elegans and zebrafish

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Due to the increasing evidence of their potential contribution to antibiotic resistance and endocrine-disrupting effects, triclosan(TCS) and triclocarban(TCC) as antimicrobials were recently banned by FDA from personal care products in the U.S. Manufacturers have switched to alternative compounds including benzalkonium chloride (BAC), benzethonium chloride (BEC), and chloroxylenol in their antibacterial products. However, the environmental and human safety of these replacement compounds have not been adequately studied. This study investigated the toxicological effects of these three replacement compounds in two model organisms, the nematode *C. elegans* and zebrafish ((*Danio rerio*), and compared them to the toxicity effects of TCS and TCC. For the nematode, both apical endpoints including lethality, reproduction, and lifespan and transgenic strains targeting oxidative stress response and germline toxicity were used. For zebrafish, developmental toxicity was evaluated using hatching rate and embryonic/larval lethality and deformities. Potential impact of these compounds on the embryonic zebrafish nervous system was also assessed by immunostaining of motor neurons. We found the three replacement compounds all induced toxicity effects in *C. elegans* at mg/L levels, with the BAC being the most toxic and showing effects on the worm reproduction and lifespan at concentrations as low as 0.1 m/L. In addition, they all induced oxidative stress and germline toxicity in the worm at sublethal concentrations. In the fish, BAC again showed the greatest toxicity among the three compounds, with effects on hatch rate, embryonic/larval mortality, and deformities at concentrations as low as 0.5 mg/L. Chloroxylenol induced significant malformation including cardiac edema and body curvature at lower mg/L concentrations, which was not observed in the other two compounds. Chloroxylenol also induced defects in motor neurons in larval fish whereas the other two did not. A comparison of toxicity effects between the replacement compounds and the original TCS and TCC suggested that these replacement compounds are not necessarily less toxic than the banned compounds. These findings demonstrate that more thorough toxicological studies are urgently needed to further understand the potential environmental impact and human health implications of these replacement compounds.

656 Substituted Diphenylamine Antioxidants and Benzotriazole UV Stabilizers in Aquatic Organisms in the Great Lakes Region

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Substituted diphenylamine antioxidants (SDPAs) and benzotriazole UV stabilizers (BZT-UVs) are organic contaminants of emerging concern, but little is known about their bioaccumulation in wild biota. The present study investigates the spatial variations, tissue-specific distributions and biomagnification profiles of SDPAs and BZT-UVs in organisms from the Great Lakes region for the first time. In an urban creek in Ontario, higher levels of SDPAs were detected in the organisms from downstream of the urban area compared with the upstream, implying a possible influence of the industrial activities on the anthropogenic antioxidant contamination in the sampling area. Benthic invertebrates and bottom feeding fish had larger body burdens of SDPAs and BZT-UVs compared to pelagic fish in the creek and liver was the major target site of these contaminants in fish. In Great Lakes, herring gull (*Larus argentatus*) eggs accumulated much higher levels of SDPAs compared to lake trout (*Salvelinus namaycush*) and the compositions of SDPAs were different in the two species. Dioctyldiphenylamine was consistently detected at low concentrations in the top predator lake trout; however, this compound showed growth dilution in trout and trophic dilution in the trout food web in Lake Superior.

657 Effects of a benzotriazole UV stabilizer and hindered phenol antioxidant on amphibians during their aquatic life stages

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The transition from tadpole to frog (metamorphosis) and sex differentiation are tightly controlled by the endocrine systems including hormones, where exposure to even low concentrations of certain environmental contaminants can change normal hormone processes that have severe effects on biological development and thus the production of healthy, reproductively capable individuals. We evaluated the sex-steroid and thyroid disrupting potential of high-risk substances identified on Canada's Chemicals Management Plan (CMP) priority list as suspected endocrine receptor binders. We assessed the toxicity and tissue residues of a plastic additive, benzotriazole UV stabilizer (CAS: 3147-75-9, UV329), and the hindered phenol antioxidant, 2, 4-*Di-tert* butylphenol (CAS: 96-76-4) in a laboratory model amphibian species, *Silurana (Xenopus) tropicalis* and a native species, *Lithobates pipiens*. These substances are used in consumer products and primarily enter the environment via waste water treatment effluents and the application of biosolids. First, we conducted standard 96 h acute toxicity tests, following the FETAX protocol (ASTM, 2012) to establish lethal and sub-lethal acute effects for these chemicals. We then used this information to select sub-lethal concentrations for chronic exposures to detect effects on growth, development and sex differentiation in *L. pipiens*. The lethal concentration that resulted in 50% mortality (i.e., LC50) for *S. tropicalis* embryos exposed to 2,4-*Di-tert* butylphenol was 0.49 mg/L [95% CI, 0.45, 0.52]. We found *S. tropicalis* hatchlings exposed to 2,4-*Di-tert* butylphenol at 0.001, 0.01 and 0.05 mg/L were significantly larger compared to controls (GLMM, all $p < 0.001$). We noted axial abnormalities, edemas/blistering, malformed eyes and mouth, abnormal head, gut, heart and tail development at concentrations ranging from 0.028 to 0.093 mg/L (R^2 ranging from 0.5024 to 0.8129). We found *L. pipiens* embryos were not sensitive to the benzotriazole UV stabilizer when exposed to concentrations from 0.0001 to 0.02 mg/L. Finally, *L. pipiens* tadpoles were chronically exposed for 30-days to sub-lethal concentrations of these compounds (ranging from 0.05 to 0.1 mg/L) and growth, development and sex differentiation were assessed. Our study supports the hazard profile risk assessment of new and existing CMP3 priority substances by providing some of the first aquatic vertebrate toxicity data for these compounds.

658 Transgenerational effects of early life stage exposure to endocrine disruptors on reproduction and development in an estuarine fish

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It has been well established that endocrine disrupting chemicals (EDCs) agonize, antagonize, or synergize the effects of endogenous hormones, affecting gene expression, causing physical abnormalities, and reducing fecundity. Newer research demonstrates that EDCs can cause deleterious effects in adulthood as a result of early-life exposure, as well as transgenerational effects. There are a paucity of studies at environmentally relevant concentrations in non-model fish species, such as Menidia beryllina, a euryhaline fish with short generation time that is found throughout North America and is demonstrated to be sensitive to contaminants. As such, we exposed *Menidia beryllina* embryos (8 hpf) until 21 dph to an androgenic and estrogenic EDC of emerging concern: levonorgestrel (Levo) (10 ng/L), bifenthrin (Bif) (5 ng/L), respectively, and coupled this exposure with testing of an established androgenic and estrogenic EDC: trenbolone (TB) (10 ng/L), and ethinylestradiol (EE2) (5 ng/L). We are now evaluating the potential for transgenerational EDC effects across three generations, with EDC exposure isolated to the parental generation (to 21 dph) only. To assess the effects of endocrine disruptors across multiple biological scales, this study is examining the potential for changes in gene expression, DNA methylation, histological analysis of reproductive organs, as well as alterations in fecundity, sex ratio, morphology, and immune response in the F0, F1, and F2 generations. Results from the first generation show that exposure to all EDCs except for EE2 significantly reduced fecundity in the parental generation. Conversely, only EE2 skewed sex ratios (feminized) relative to controls. Findings from F0 and F1 generations suggest that exposure to EDCs increased growth in the parental generation, and that androgenic treatment groups (Levo, TB) maintain this growth through the subsequent F1 generation. In the parental generation, differences in adult immune response are apparent between bifenthrin and levonorgestrel. Future data gathered on gonadal histology, gene expression and DNA methylation will allow us to further hone in on the mechanisms causing higher order downstream effects. Elucidation of the mechanisms of these higher order downstream effects will allow for quantification of effects of exposure to endocrine disruptors across multiple biological scales, and for a comparison between a range of responses to emerging and established EDCs.

659 Halogenated Chemicals Affect Thyroid and Estrogenic Signaling in Japanese Medaka

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Halogens can act as endocrine disrupting chemicals (EDCs) altering thyroid function as well as estrogenic signaling. Examples of these chemicals include perfluorinated chemicals used in Teflon and food packaging, and flame retardants, used in clothing and electronics. Exposure of aquatic organisms to these EDCs can result in delayed growth and development. However, little is known regarding gender differences in these effects. Japanese medaka offer several advantages for the study of gender-specific putative EDCs including the ability to differentiate gender at an early age as well as the presence of only one swim bladder that is fully developed at the time of hatching which is an excellent organ to study for thyroid toxicity. Two well-known hazardous EDCs, perfluorooctanoic acid (PFOA) and tris (1,3-dichloro-2-propyl) phosphate (TDCPP) and a next generation chemical, perfluorobutyric acid (PFBA), were tested for their potential thyroid-estrogenic disruptive potential. We measured effects on swim bladder (SB) development as well as the expression of a suite of thyroid and estrogen responsive genes including thyroid stimulating hormone beta, thyroid receptors alpha and beta, surfactant proteins A, B and C, estrogen receptors alpha and beta and vitellogenin (*tshβ, trα, trβ, spA, spB, spC, esrα, esrβ and vtg*). We observed an over inflation of the SB, but only in females, strongly suggesting gender differences in SB dysfunction. Males showed increased estrogenic gene expression after exposure to chemicals. Upregulation of thyroid related genes were observed in both genders. Overall the next generation chemical appeared to be less toxic than their more toxic counterpart due to less severe swim bladder effects as well as no differences in gene expression.

660 Flame retardant effects on water quality, primary producers and macroinvertebrates in artificial streams

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Flame retardant chemicals are now pervasive in aquatic systems due to widespread industrial use and subsequent discharge in municipal and industrial wastewater. Current information about the toxicity of organophosphorus flame retardants, such as tetrakis hydroxymethyl phosphonium chloride (THPC), is derived primarily from acute laboratory tests on individual species. However, THPC degrades slowly and has the potential to persist in the environment with chronic effects. Furthermore, regular discharge into stream environments can be particularly detrimental to downstream benthic biota with little or no mobility. The goal of this study was to assess the influence of THPC on water quality and benthic communities in artificial streams over a two-week period during the summer of 2016. Water column pH, ion concentrations, periphyton biomass and chlorophyll *a* content, phytoplankton chlorophyll *a*, and macroinvertebrate colonization were measured following exposure to 0, 0.08, 0.8 or 8 mg L^{-1} THPC. Addition of THPC affected pH; in the presence of 0.8 mg L^{-1} pH was elevated while the pH in the 8 mg L^{-1} treatment decreased relative to the control. However, these changes were not likely to affect the biota because pH remained between 7.6-7.9, regardless of treatment. The concentration of chloride, a degradation product of THPC, was ~2 mg L^{-1} higher in the 8 mg L^{-1} THPC addition compared to all other treatments. The presence of THPC decreased periphyton biomass in all treatments by 43-66% compared to the control, but periphyton chlorophyll *a* concentration did not differ. Phytoplankton chlorophyll *a* and macroinvertebrate colonization were not affected by THPC addition over the two-week experiment. These findings suggest periphyton is more sensitive to THPC than macroinvertebrates in stream communities under environmental conditions. Moreover, periphyton loss has the potential to affect macroinvertebrate food supply and subsequently alter aquatic community composition over longer time frames.

Next Generation Nanotechnology – Environmental Health and Safety of Nano-enabled Products and Advanced Materials

661 From nano to convergence: The growing divide between innovation and risk assessment

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As predicted by Roco et al, the fruits of nanoscale science and engineering continue to increase in sophistication, from one-dimensional nanostructures to higher ordered advanced materials systems. Within these systems, nanotechnology may be but a single component of multiple converging technology domains including biotechnology, big data, artificial intelligence, autonomous systems, and others. Each domain brings unique risks that must be characterized independently, upon integration with one or more of the other domains, and upon introduction to biological and environmental systems. The last decade of nanoecotoxiology and fate/transport research illustrated some of the challenges posed by converging technology risks. Among those challenges were the introduction of nanoscale structure, the potential for dissolution and aggregation, interactions with biological and environmental macromolecules (e.g., sorption of proteins or natural organic matter), and interactions with other chemicals (some of which may have enhanced transport characteristics when sorbed to nanoscale materials). Increasing system complexity will bring new boundaries that are certain to challenge basic nomenclature, prioritization of research needs, analytical approaches, bioassays, risk assessment frameworks, and regulatory approaches. The interdisciplinary questions that arise where technology domains intersect provide a glimpse into the risk assessment challenges that lay ahead. Much like our assessment of single-dimensional nanostructures was built upon decades of experience with chemicals, our ability to assess risks of advanced materials can build upon our improved understanding of engineered nanomaterials. Ultimately, the accelerating pace of innovation and the rapid introduction of convergence-enabled advanced materials to consumer markets will exacerbate our ability to assess and manage risks. Whether and to what extent current risk assessment capabilities can keep pace with 21st century innovation will define the human and environmental health and safety challenges of the future. This presentation will explore how lessons learned from nano can help inform and enhance efforts to assess and mitigate risks posed by the new generations of advanced materials enabled by converging technologies.

662 Current Approaches for Assessing Ecological Hazard of Carbon Nanotubes: USEPA Regulatory Perspective

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The Frank R. Lautenberg Chemical Safety for the 21st Century Act was signed into law on June 22, 2016, thereby amending the Toxic Substances Control Act (TSCA), the Nation's primary chemicals management law. Implementation of the amended legislation is carried out by USEPA's Office of Pollution Prevention and Toxics (OPPT) and includes assessments of both existing and new chemical substances in commerce. Of approximately 1,000 new industrial chemicals submitted to USEPA for review each year, approximately 1% are engineered nanomaterials (ENMs). Over 190 ENMs submissions have been received in the New Chemicals review process since 2005. The majority of materials seen thus far are carbon nanotubes (CNTs). For the vast majority of submitted

CNTs, little or no ecotoxicological information is provided to USEPA, which presents challenges to the risk assessment/management process. Quantitative structure activity relationships (QSARs) are commonly used to address these uncertainties for organic chemicals, but CNTs do not follow the same equilibrium partitioning behavior that applies for many organic chemicals, therefore partitioning parameters (e.g. K_{ow}) are not appropriate for predicting CNT toxicity. Currently, USEPA ecological risk assessors use analog information, when available, as well as weight-of-evidence approaches and professional judgement to assess the potential ecological hazard of CNTs. Hazard approaches will be strengthened as more ecological test data become available on CNTs (in the open literature as well as supplied in new chemical application submissions). This presentation will discuss current ecotoxicological hazard approaches for CNTs, their strengths and limitations, and touch on current data gaps/needs.

663 Properties and hazard uncertainty are bigger than size: Insights from a tiered framework for advanced (and nano-enabled) materials

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Advanced Materials (AdMs), including nanomaterials (NMs), offer novel properties that promise to enhance conventional materials science in exciting ways that should be fostered. However, given their uncertain Environmental Health and Safety (EHS) risk profiles, many AdMs and NMs may receive higher prioritization for regulatory testing under the reformed Toxic Substances Control Act (TSCA), now the Lautenberg Chemical Safety Act (CSA). The CSA seeks to streamline the decision process for regulatory acceptance of new substances through tiered testing strategies that offer step-wise approaches to EHS assessment that are practical and cost efficient. We developed a tiered testing framework focused on first classifying technologies by their novel properties (or uses) that relate to unknown hazards. The tiered strategy was previously demonstrated using i) a nano-thermite energetic application and ii) a nano-silver printable circuit. This prior work highlighted the ambiguity of size-only definitions since the hazard profiles of these NM-enabled technologies were driven by ion release, and their fate/transport was governed primarily by sintered, micron-sized aggregates. These findings prompted a transition from a size-based definition of AdMs, to a properties-based definition stated as follows: "*materials intentionally engineered to exhibit novel or enhanced properties that confer superior performance relative to conventional materials"*. In this presentation, we discuss case studies demonstrating classification of AdMs defined by: i) both unique properties and nano-scale size (photocatalytic TiO_2 cement, nanotube-sensors); ii) combination of several conventional materials into a novel application (cellulose-based hydrophobic combustible casing for energetics); and, iii) novelly manufactured 3-D printed munitions (energetic materials). In the case studies, different use-relevant release tests were considered. Initial results indicate that the classification scheme and tool (called NanoGRID) were effective for prioritizing a large number of testing possibilities to a more managable suite of testing, based upon pre-defined parameters (relevance, worst case scenarios, expertise, time).

664 NanoPHEAT a Nano Product Hazard and Exposure Assessment Tool

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In the last decade, the production and use of NMs-containing products have increased dramatically, and that has raised concern about potential impacts of NMs on human health and the environment. Exposure studies of NMs released from product show different speciations of NMs released to various environmental compartments: either dissolved, fully or partially embedded in matrix residues, hetero-agglomerated or free. Apart from the influence of the environmental media on NM fate, the behavior and toxicity of NMs as released from a product can be driven by the product matrix property (i.e. fully or partially embedded in matrix residues). The embedding of an NM within a product not only controls the NM exposure, but also has implications on the NMs toxicity. While the difference between pristine and realistic NM exposure and toxicity is critical to consider. For every product associated with a weathering scenario we define a unitless "matrix factor" refering to the fraction of observed nano-enabled weathered product toxicity vs. the pristine nanomaterial. This fraction captures the grouped effects of altered exposure, bioavailability and bioactivity related to NM incorporation and release from product matrices, and informs forecasts of potential risks to the environment and consumer. In the study, we will present the NanoPHEAT model (Nano Product Hazard and Exposure Assessment Tool). The model is built to compile toxicity dose-response curves of pristine NMs compiling hundreds of published records, the calculates and superimposes the estimated exposure to nanoparticles as released from real products as measured via in-house experiments, as well as some emerging product release literature. We firstly focus on a nanocomposite incorporating CNT-based materials that we supposed to have a high matrix ratio. We incorporated different CNT in the bulk of 3D polymer printed (PETG) in order to increase product conductivity and strength. After simulation of materials aging, we quantify the fraction of CNT release and bioavailable using an acid digestion CNT catalyst aggressive but PETG friendly in order to quantify the CNT release fraction that will be in contact to the environment. In parallel, the 3D polymer printed residue of degradation toxicity was test tested toward fish. This matrix factor derived from this experiment is then applied within the NanoPHEAT model to forecast expected consumer toxicity against a curated mammalian toxicity dataset.

665 Issues in hazard testing of nanomaterials and nano-enabled products: Nano-specific versus physical effects and exposure-response dosimetry

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The size and particulate nature of pristine nanomaterials (NM) introduce complexities for determining their hazard in aquatic systems. These include potential nanoscale-specific mechanisms of toxicity (e.g. arising from quantum processes), release of toxic constituents (e.g. dissolution of nano-silver), particle or fiber driven physical effects, and exposure-response determination. We present here examples of these issues for pristine, photocatalytic nano-TiO_2 (pn-TiO_2) and a nano-enabled product (NEP) that incorporates pn-TiO_2; photocatalytic cement. This NEP can be considered an advanced material due to its size facilitated enhanced photocatalytic properties, and also represents NM applications in which the target function equates directly with a known toxic mode of action. In our testing, we have confirmed (in 48-h *C. dubia* bioassays) results of previous studies demonstrating the 10-fold to 1000-fold increase in toxicity of pn-TiO_2 when tests are conducted under simulated solar radiation (LC50 = 2.54, c.i. 2.20 – 2.94 mg/L, vs. LC50 = 0.033, c.i. 0.026 – 0.042 mg/L and 0.036, c.i. 0.031 – 0.043 mg/L (in separate replicate assays), respectively. Separate test of particulate pn-TiO_2 also revealed a delayed toxic response, apparently driven by initial phases of exposure, and suggesting a physical mode action. We have also tested pn-TiO_2 as it is used in commercially available concrete, where its photocatalytic activities render concrete surfaces "self-cleaning" and "depolluting". Testing revealed no increase in toxicity of pn-TiO_2 cement tested in either as-delivered or abraded (from prepared mortar samples) form, compared with control Portland cement (no TiO_2 added), and under laboratory lighting or simulated sunlight. LC50's for these materials ranged from 3.49 (c.i. 1.57-6.00) mg/L (as-delivered, control Portland cement), to 37. 4 (c.i. 18.28-55.36) mg/L (as delivered pn-TiO_2 cement). These materials required novel approaches to exposure monitoring and response dosimetry, requiring use of time weighted averaging techniques that revealed an approximate two-fold error in determination of effect levels when material losses were not quantified. Dosimetry was further complicated by the complex matrix of cement and pn-TiO_2 that comprise particles, and the unknown proportion of pn-TiO_2 present on particle surfaces. It is likely that these issues will be exacerbated by the increasing complexity of NEPs, and may also apply to other advanced materials.

666 Approaches for Dose Verification in Aquatic Ecotoxicology Testing of Engineered Nanomaterials

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Engineered nanomaterials (ENMs) are increasingly used in manufacturing processes and as additives to consumer products to enhance product properties. Understanding the environmental fate and impact of ENMs relies on the ability to characterize them at relatively low levels in complex media, which often contains additional naturally occurring nanomaterials. We present the application of single-particle inductively coupled plasma mass spectrometry (sp-ICP-MS) and a recently developed hyphenated technique combining electrospray aerosol generation (ESI), particle size selection using differential mobility analysis (DMA), and elemental analysis using inductively coupled plasma mass spectrometry (ICP-MS) to characterize gold and silver nanoparticles in aqueous media. These methods are capable of detecting nanomaterials of 20-100 nm diameter at part-per-billion (ppb) levels in environmentally relevant aqueous solutions. Following instrument characterization that utilized NIST standard reference materials (SRMs) and other well characterized materials, the methodology was used to assess the fate of silver and silica dioxide nanoparticles in a solution containing daphnia magna. Insights into dose verifications obtained by the method will be discussed, as well as the analytical performance of the method.

667 Multiple method comparison of nanoparticle concentration measurements

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One key measurement for dispersed engineered nanomaterials (ENMs) is the nanoparticle number concentration. This has been proposed for potential use in regulatory toxicity testing and as a potentially more important metric than the mass based concentration for nanotoxicological studies. In this study, we compare nanoparticle number concentration measurements of four gold nanoparticles dispersed in aqueous suspension using multiple methods: single particle inductively coupled plasmamass spectrometry, nanoparticle tracking analysis, differential mobility analysis, dynamic light scattering, and scanning electron microscopy. For the techniques that provided an ENM size distribution but not a direct measurement of the nanoparticle number concentration, total gold elemental concentrations were used to calculate the nanoparticle number concentration. The feasibility of using nanoparticle number concentration in standard ecotoxiciology assays will be discussed.

668 Bioaccumulation and depuration of nanoparticles in replicated outdoor wetland mescosms vary with particle size, particle type and duration of exposure

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Engineered nanomaterials such as manufactured nanoparticles (NPs) are ever more widely produced, used and released into surface waters, which may pose a risk to human and ecosystem health. While there is a growing body of research on the toxicity of NPs, risk assessment must account for the bioavailability of NPs at environmentally relevant exposures and in complex ecosystems. Here, we present results from a study in replicated outdoor wetland mesocosms on the bioavailability of two cerium dioxide (CeO_2) NPs of widely divergent sizes (small NPs ~3.8 nm, large NPs ~185.3 nm) in eastern mosquitofish, *Gambusia holbrooki*. We hypothesized that a longer exposure duration in this system would increase the bioaccumulation and lower clearance of NPs in the fish, especially for the small NPs compared with the large NPs. Our aims were to test this hypothesis and understand mechanistically the route of exposure and absorption-distribution-excretion processes by quantifying the NP uptake rate and depuration kinetics in fish exposed for either long-term (i.e. repeated weekly NP pulse exposures over 6 and 9 months) or short-term (i.e. one NP pulse exposure over one week) durations. ICP-MS results in fish homogenates indicate that small CeO_2 NPs were taken up more readily than large NPs by the fish in short-term exposures. However, there was not a NP size difference and both NPs were readily taken up in long-term exposed fish. Surprisingly, there was a higher degree of clearance of the small CeO_2 NPs than the large NPs for short-term exposures. However, over just a 24 h depuration period the long-term exposed fish had a full clearance of CeO_2 NPs (to background level), to a significantly higher degree than the short-term exposed fish, counter to our hypothesis. Ongoing efforts are focused on comparing these results to the bioavailability of gold (Au) NPs of an intermediate size (i.e. between the small and the large CeO_2 NPs) as another stable nanoparticle and adding additional aquatic taxa. Preliminary analyses from the same experiments for Au NPs indicate that the long-term exposed fish have a higher concentration of Au NPs and have a much lower clearance over 24 h than the short-term exposed fish. This fits our original hypothesis, but is counter to our CeO_2 NP results, highlighting the importance of accounting for depuration in bioavailability and that some processes are not generalizable to different NPs.

Fate and Effects of Metals – Regulatory and Risk Assessment Perspective

669 How does information about aluminum bioavailability inform what we measure to compare with aluminum water quality criteria?

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Aluminum (Al) bioavailability and toxicity to aquatic organisms is strongly affected by water chemistry. In particular, pH, dissolved organic carbon (DOC), and hardness can change the observed toxicity of Al to aquatic organisms resulting in wide variation in observed effects. The BLM framework for metals considers how water quality can impact the chemical speciation of dissolved metal and the interaction of metal ions with biological surfaces, thereby resulting in changes in metal bioavailability. A review of Al toxicity data, however, shows that concentrations of Al sufficient to cause toxicity are frequently in excess of solubility limitations. Al solubility is strongly pH dependent, with a solubility minimum near pH 6 or 7, depending on temperature and other water chemistry characteristics (e.g. DOC concentrations). Conceptually, the BLM framework should be a valid description of metal bioavailability when toxicity is a result of exposure to dissolved Al, but the mechanistic framework needs to be extended to allow toxicity from a combination of dissolved and precipitated Al. These unique aspects of Al bioavailability suggest that accurate characterization of bioavailable Al cannot simply be based on dissolved Al concentrations. On the other hand, Al is naturally occurring and abundant and can account for a significant percentage by weight of suspended particles that are captured in surface water samples. As a result, total Al measurements in natural water samples are likely to result in sufficient contribution from natural particles that exceedances of water quality criteria are frequent whether or not there are bioavailable or anthropogenic sources of Al. Exceedances, of water quality criteria based on total Al measurements, therefore, are often false positives that do not indicate impairment. In this presentation, we will show an analysis of surface water Al concentrations, the association between Al and various particle size fractions, and a comparison of measured Al with theoretical solubility limits. This analysis suggests that most of the Al measured in surface waters is associated with naturally occurring non-toxic and non-bioavailable Al minerals, and that monitoring programs designed to characterize Al concentrations for comparison with Al water quality criteria should consider both the natural occurrence and bioavailability.

670 Limits to the Application of Updated Aquatic Life Protection Criteria for Metals Resulting From Antidegradation Review: A Cautionary Tale

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Recent scientific research has generated the data needed to update regulatory aquatic life protection criteria for several metals (e.g., copper, aluminum, and zinc). As a result, these criteria now take into account the toxicity mitigating effects of water hardness, pH, and dissolved organic carbon (DOC), often generating criteria concentrations that are substantially higher than the older hardness-based criteria. Even though these updated criteria concentrations can be higher, use of USEPA guidelines for criteria derivation ensure that the levels of intended aquatic life protection are the same. However, it is becoming increasingly apparent that Clean Water Act-mandated antidegradation and anti-backsliding policies may limit the applicability of updated metals criteria by preventing increases to regulated effluent limits or loads even if the underlying water quality standard is changed. This is because antidegradation and anti-backsliding policies are based on the premise that increased pollutant loads equate to increased “degradation” to aquatic life uses. In contrast, in situations where metals criteria increase under conditions of low bioavailability (e.g., elevated pH, hardness, or DOC), the amount of aquatic life “impact” is not any greater so long as the criteria are derived using levels of protection set forth under USEPA guidelines. This presents a regulatory roadblock to implementation of new metals criteria derived using the most recent data and scientific methods in situations where water quality conditions support higher criteria concentrations than those derived using older methods (e.g., using hardness alone). Here we present some case studies illustrating the limits this can place upon the application of new metals criteria (using copper as an example), and explore options for how antidegradation and anti-backsliding policies could be applied in such a way as to reflect the latest science, and still provide the intended levels of aquatic life protection.

671 Perceived Impacts of the Over-Representation of Sensitive Taxa in Sensitivity Distributions

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Since the mid-1970s, thousands of studies have evaluated the toxicity of various chemicals to aquatic organisms. Results from many of these studies have been used to develop species sensitivity distributions (SSDs) or genus sensitivity distributions (GSDs) for deriving water quality guidelines. Recently, there has been more emphasis on evaluating the toxicity of chemicals to sensitive organisms rather than the entire range of sensitivities. The SSD is intended to inform the derivation of guidelines for the protection of all species, not just those that were included in the SSD. The over-emphasis of the more sensitive end of the SSD can contribute to a skew in the observed distribution such that the shape of the distribution is distorted from what it would be if all species could be tested, which ultimately affects the derived guideline value. The freshwater acute copper GSD derived by the U.S. Environmental Protection Agency is one that exemplifies this trend, with one-third of the genera in the GSD belonging to only three taxonomic families, all of which are nearer to the sensitive end of the distribution. The stronger representation of the more sensitive families does not seem to mirror the overall abundance of species within those families in nature. This tendency towards testing sensitive organisms is not seen in the chronic copper SSD. This trend towards collecting data for sensitive taxa is more pronounced in the acute copper toxicity data since it is a well-studied chemical, but the issue could extend to other toxicity datasets now or in the future. In this presentation, we will review the copper toxicity literature and examine long-term trends in the availability of toxicity information for species of varying sensitivity. As part of this review, we will demonstrate what appears to be a bias that favors the publication of toxicity data for sensitive taxa, and evaluate the influence of this apparent bias on a water quality guideline depending on the derivation method used (i.e., fitting the full distribution or truncating to the most sensitive organisms). Implications for the representativeness of SSDs and their use in developing water quality guidelines will be discussed.

672 Protectiveness of Copper Water Quality Criteria Against Impairment of Behavior and Chemo/Mechanosensory Responses: An Update

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To update and expand on previous analyses from almost a decade ago, we conducted a meta-analysis of studies that reported behavior and chemo/mechanosensory responses by fish, amphibians, and aquatic invertebrates in Cu-containing waters and that also reported sufficient water chemistry for calculation of the U.S. Environmental Protection Agency’s former hardness-based and current biotic ligand model (BLM)-based water quality criteria (WQC) for Cu. The calculated WQC concentrations were then compared to the corresponding 20% impairment concentrations (IC20) of Cu for those behavior and chemo/mechanosensory responses. The hardness-based acute and chronic WQC for Cu would not have been protective (i.e., the IC20 would have been lower than the WQC) in 33 and 26%, respectively, of the 106 combined behavior- and chemo/mechanosensory-response experiments that had adequate water chemistry data for hardness-based and BLM-based WQC calculations. In comparison, the BLM-based acute and chronic WQC for Cu would not have been protective in only 9 and 4%, respectively, of the same 106 cases. In many of the experiments in which the BLM-based WQC would not have been protective, either (1) the Cu did not appear to have been adequately equilibrated with the dissolved organic matter (DOM) in the exposure waters or (2) only nominal (i.e., not measured) Cu concentrations were reported. To improve evaluations of regulatory effectiveness, researchers conducting aquatic Cu toxicity tests should measure and report complete BLM-input water chemistry, bracket the hardness-based and BLM-based WQC concentrations for Cu that would be applicable in their exposure waters, and ensure that adequate time is allowed for Cu to equilibrate with the DOM in exposure waters before starting an experiment. This meta-analysis demonstrates that overall, the BLM-based WQC for Cu were considerably more protective than the hardness-based WQC against impairment of behavior and chemo/mechanosensory responses.

673 Update on Aluminum Ambient Water Aquatic Life Criteria

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The US Environmental Protection Agency (EPA) is updating its 1988 recommended aquatic life criteria for aluminum under Clean Water Act Section 304(a). The 1988 criteria account for the influence of pH on toxicity, similarity of acute toxicity of fish and invertebrates, and the greater sensitivity of invertebrates in chronic toxicity tests. The 1988 document recommended an acute criterion of 750 µg/L and a chronic criterion of 87 µg/L when the pH is between 6.5 and 9.0. The effects of hardness were not considered in the 1988 criteria derivation. Over the past 25 years, new acute and chronic toxicity data for both freshwater and saltwater organisms have been published. Additional toxicity data for the eight recommended families and mussel toxicity studies are included in the new evaluation. There were a variety of technical approaches considered for criteria calculation. EPA compared several approaches that reflect water quality condition impacts on toxicity including complete and simplified aluminum Biotic Ligand Models (BLMs) and multi-linear regression equations using pH, hardness, and dissolved organic carbon parameters. Comparisons were conducted to facilitate evaluation of the various approaches for criteria development. The multi-linear regression approach using pH, hardness, and dissolved organic carbon parameters was selected for criteria development due to 1) the transparency of the model, 2) the similarity and accuracy of the results relative to the available BLM model outputs and empirical data, and 3) the decreased number of input water chemistry data needed to derive criteria at different sites, which increases utility of the model for end-users.

674 Federal Water Quality Guideline for Copper (Canada)

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Environment and Climate Change Canada (ECCC) develops federal water quality guidelines (WQGs) for the protection of aquatic life for metals. ECCC uses CCME (2007) methods to the extent possible. Depending on the availability of toxicological data and influence of toxicity modifying factors (TMFs) several approaches are possible. The draft federal water quality guideline (FWQG) for copper uses the biotic ligand model (BLM) approach that involved compilation and evaluation of chronic copper toxicity data, evaluation of model performance, utilization of the BLM to normalize the toxicity data to site-specific conditions and application of species sensitivity distribution (SSD) models. The underling approach is similar to the USEPA and EU BLM efforts; however, the differences result from the parameters considered, the toxicity database and the normalization of toxicity data in SSD. The approach is similar to that of the EU in that it is based on chronic toxicity data, considers plants in addition to fish and invertebrates and utilizes multiple BLM parameters. However, the approach differs from the EU in that it uses a full BLM approach for plants rather than a regression on free copper ion. The approach is also similar to the USEPA in that a single set of BLM parameters are used for all fish and invertebrate species. A user-friendly BLM tool has been developed for calculating the site-specific FWQGs with option of using detailed or simplified site chemistry.

675 It's not a hard choice: Comparing hardness- and bioavailability-based Water Quality Criteria approaches for nickel

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The European Union (EU) has established a bioavailability-based Environmental Quality Standard (EQS) for nickel (Ni) under the EU Water Framework Directive (WFD). The bioavailability correction for the Ni EQS is performed using chronic Ni Biotic Ligand Models (BLMs), which account for the influence of hardness, pH, and dissolved organic carbon (DOC) on Ni toxicity. As is the case with many jurisdictions, the current USEPA Ni Water Quality Criteria (WQC) for protection of aquatic life is based on hardness correction alone. To explore the impact of applying full bioavailability normalization in the determination of a Ni WQC for US surface waters, we applied currently available Ni BLMs to a chronic ecotoxicity database using water chemistry parameters obtained from a US Geological Survey monitoring database. We also applied the USEPA hardness equation to the same aquatic ecotoxicity data to compare hardness- and bioavailability-based approaches. Results indicated that the hardness normalized values can both over- and under-estimate Ni toxicity. This likely reflects the influence of pH and DOC on Ni toxicity, which can only be accounted for when full bioavailability normalization is performed. The discrepancy between hardness- and full bioavailability-normalized outcomes is highlighted in two scenarios: 1) low hardness, low pH and high DOC systems that occur in Northern Forests, for which hardness-based values are low and where bioavailability normalized values are high; and, 2) high hardness, alkaline pH, and low DOC systems that occur in various parts of the US for which hardness-based values are high and where bioavailability normalized values are low. Results of this analysis will contribute to USEPA's consideration of bioavailability based approaches for metals like Ni.

676 Influence of natural dissolved organic matter on chronic toxicity of aluminum to Ceriodaphnia dubia in soft test waters

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The toxicity of aluminum (Al) to freshwater biota varies widely due to Al's complex pH-dependent speciation. Recent draft water quality criteria estimate protective criteria for Al based on pH differences (low, 5.0-6.5; high, 6.5-9.0) and empirical regressions of Al toxicity versus water hardness. Implementation of updated water quality criteria should consider factors other than hardness that may modify Al toxicity, especially in soft-water systems. Streams draining watersheds with resistant bedrock, which are common in areas such as the Appalachian region of New England and the Canadian Shield region of the Great Lakes states, often have limited buffering capacity, low pH, low hardness, and high background Al concentrations that could lead to restrictive Al criteria/standards, and frequent exceedances of hardness-based criteria. We investigated the influence of natural dissolved organic matter (DOM) on Al toxicity to *Ceriodaphnia dubia (C. dubia)* by conducting a series of 7-day/3-brood chronic tests. Test waters consisted of serial dilutions of two soft natural waters collected from sites in Massachusetts, USA, with original dissolved organic carbon (DOC) concentrations of 6 and 10 mg/L and our laboratory well water (lab water; DOC < 0.4 mg/L; hardness 300 mg/L as $CaCO_3$) diluted with deionized water to hardnesses of 20 and 35 mg/L as $CaCO_3$. Site waters were mixed with lab water to produce test waters containing 100%, 50%, and 25% of the original DOC concentration. Tests were conducted in an incubator with a controlled CO_2 concentration to maintain pH in the target range of 5.8-6.5. Results of these tests were used to estimate chronic toxic effect concentrations for *C. dubia* mortality and reproduction. Effect concentrations for *C. dubia* increased linearly across nominal DOC concentrations up to 10 mg/L, but there were no detectable differences in thresholds between waters with 20 and 35 mg/L hardness. Thresholds for total Al averaged 1.21 mg/L in lab water (0.33 mg/L DOC) and 5.5 mg/L in waters with low concentrations of natural DOM (DOC 1-2 mg/L). This increase in the total Al toxicity threshold due to moderate increases in the DOC resulted in a greater range of toxicity than that predicted by the draft criteria for the entire hardness range, 0-300 mg/L. These results indicate that water quality criteria for total Al based on hardness alone are likely to be overprotective for *C. dubia* and perhaps for other aquatic taxa in soft, low-pH waters.

Impacts of Sulfate Inputs to Freshwater Ecosystems in the Great Lakes Region

677 Sulfate pollution enhances organic decomposition and releases N, P, and Hg

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Bacterial sulfate reduction in sediment is stimulated by increases in sulfate in the overlying surface water. It has not been generally recognized that enhanced sulfate reduction is necessarily associated with enhanced decomposition of organic matter, which releases the constituents of the organic matter. We present the results of a sulfate-addition experiment to outdoor mesocosms that demonstrates the release of carbon, nitrogen, phosphorus, and, surprisingly, mercury to the overlying surface water. Carbon releases result in increases of both alkalinity and dissolved organic carbon (DOC). Consequently, sulfate pollution has the potential to fundamentally shift a freshwater ecosystem toward a eutrophic, mercury-contaminated system with altered flora. Although sulfate is benign under aerobic conditions, the addition of sulfate causes a cascade of interrelated and potentially deleterious ecosystem consequences. Bacterial reduction of sulfate that diffuses into anoxic sediment porewater produces sulfide, which may precipitate with iron and other metals, or, in metal-poor sediments, can accumulate to phytotoxic levels. The release of phosphorus is exacerbated when sediment iron is tied up as a sulfide compound, which sorb phosphate relatively poorly, compounding the phosphorus release via decomposition and driving the system toward eutrophication.

678 Eutrophication effects of sulfate reduction in lakes and reservoirs - case studies and foundations

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Sulfate reduction is a principal forcing function of eutrophication in lakes and reservoirs. When thermal or thermo-haline stratification isolates surficial sediments from atmospheric oxygen exchange, sulfate reduction quickly follows depletion of dissolved oxygen. Sulfate reduction induces a non-linear geochemical cascade with profound ecological consequences. In stratified water basins, there is scant in-situ evidence of textbook “redox ladders” in which there is progressive depletion of higher energy terminal electron accepters prior to substantial reduction of sulfate. Rather, production of sulfide below the sediment surface induces and accelerates positive feedbacks that dissolve Fe(III) and Mn(IV). As sulfide reduction rapidly dominates redox dynamics of sediment-water solute exchange, PO_4-P, NH_4^+, Fe(II) and Mn(II) effluxes from sediments follow the same power function relationship with in-situ redox measurements. As a function of time, these effluxes are exponential. Methylmercury efflux is observed near their common asymptotes. Depletion of hypolimnetic sulfate occurs in an unambiguously inverse functional relationship with these effluxes. These functional relationships suggest that sulfate enrichment has the potential to intensify internal loading of macro and micronutrients across geographically large scales, thereby increasing eutrophication of surface waters. Because sulfate reduction increases rapidly with rising water temperatures, global warming coupled with sulfate enrichment may have adverse implications for eutrophication in the low-sulfate, temperate surface waters of the Great Lakes Region. Where internal nutrient loading dominates water quality, ecological engineering methods can reverse eutrophication by preventing sulfate reduction. Case studies of hypolimnetic injection of pure oxygen or nitrate clearly demonstrate impacts of the sulfate redox system on reservoir and lake water quality. Although economic considerations may limit these process-based management methods to high value basins, such as drinking water reservoirs, the increasing, consistent, empirical weight of these projects provides useful insights into the importance of sulfate as a master variable regulating the trophic status of surface water.

679 Interactions between sulfate and mercury methylation in freshwater ecosystems in the northern Minnesota

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Mercury (Hg) and its bioaccumulative form, methylmercury (MeHg), are common contaminants of concern in fisheries across the Great Lakes region because of neurotoxic health effects on humans and wildlife. Sulfate inputs affect the biogeochemical cycling of mercury within freshwater ecosystems through the stimulation of sulfate-reducing bacteria (SRB) and the production of sulfide as a metabolic byproduct. Sulfide is an important ligand for mercury and plays a dual role in the facilitation and inhibition of the Hg methylation, the process that creates MeHg. In the last decade, several large-scale studies in northern Minnesota examined the complex interactions between the biogeochemical cycles of sulfur and Hg in freshwater ecosystems across a gradient of sulfate inputs. Experimental manipulations of sulfate inputs to an ombrotrophic bog were conducted from 2001-2009 in the Marcell Experimental Forest; geochemical investigations of wetlands and lakes impacted by mining activities were conducted from 2012-2013 on the Mesabi Iron Range; and experimental mesocosms were used to study the effect of increasing sulfate concentrations on wild rice growth from 2011-2013. Despite variations among study systems in the time frame, size, and scale of sulfate impact (0.5-500 mg sulfate L^{-1}) the shifting influence of sulfate on Hg methylation and mobilization was consistent. Generally Hg methylation was stimulated by sulfate inputs to low sulfur systems. In high sulfate systems Hg methylation appeared to be inhibited by high levels of sulfide and additional sulfate inputs had limited effect on MeHg levels. Although seasonal variations in these trends occurred at all sites, annual patterns were consistent. Geochemical factors that specifically affected sulfide also influenced the observed relationships between sulfate inputs and Hg methylation. Concentrations of dissolved iron in stoichiometric excess of sulfide sequestered sulfide and inhibited Hg methylation. Fluctuating water levels and the introduction of oxygen inhibited Hg methylation through suppression of anaerobic SRB populations. Sulfide also governed partition coefficients for Hg and MeHg between the solid phase and porewaters because sulfide is a ligand for both. Although total Hg deposition controls the broad pattern of regional Hg contamination of ecosystems, the Hg methylation and mobilization potential of any particular ecosystem may be strongly controlled by local dynamics of sulfur biogeochemistry.

680 Assessing internal loading of methylmercury in a sulfate-impacted freshwater coastal estuary through extensive field study

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The St. Louis River Estuary (SLRE) is a large ($50km^2$), shallow, freshwater estuary that empties into the western tip of Lake Superior. The lower estuary contains the Duluth-Superior Harbor, a major inland shipping port, and boasts decades of industrial and recreational activity. The upper estuary contains many riparian wetlands and embayments and is impacted by sulfate from iron mining discharges. The SLRE contains a variety of habitats which differ with respect to water depth, residence time, sediment geochemistry, and fish assemblages. Previous work has demonstrated elevated fish tissue mercury in the SLRE compared to the upstream river and downstream Lake Superior, suggesting internal loading of bioaccumulative methylmercury. Methylmercury is produced in anaerobic sediments, primarily by sulfate-reducing bacteria as a by-product of heterotrophic metabolism. The purpose of this study is to determine the role of different habitat zones in the production and transport of methylmercury in the SLRE. Mercury and methylmercury was quantified in surface water, pore water, and sediment to understand how methylmercury is produced and partitions in each habitat zone while flux studies were conducted to estimate transport into the water column. Mercury quantity and speciation in surface water, sediment, and porewater varied across the different habitat zones. Solid phase methylmercury was highest in sediments with intermediate organic carbon content (around 10% LOI), but porewater total mercury was negatively correlated to organic carbon. Porewater methylmercury was lowest in sediment with the highest acid volatile sulfide (AVS), suggesting both inorganic and methylmercury bioavailability are partially controlled by solid phase organic carbon and sulfate. Surface waters of the isolated side embayments adjacent to riparian wetlands filled with river water during high-flow events, but had depressed DO and significantly higher methylmercury concentrations during low flow conditions, leading to surface water methylmercury concentrations being three times higher in the isolated embayments compared to the main channel. This study furthers our understanding of the drivers of methylmercury cycling in the SLRE, identifies habitats which act as sources, and attempts to place constraints on the magnitude of the internal MeHg sources relative to upstream contributions.

681 Statistical models predicting porewater sulfide from sulfate

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Elevated sulfide in sediment porewater is increasingly being recognized as a factor that may control the presence of rooted aquatic macrophytes and benthic invertebrates. With the goal of understanding the variables controlling the production of porewater sulfide in freshwater systems, we analyzed data from a large survey of shallow freshwater systems in Minnesota. Structural equation modeling (SEM) was used to identify key variables governing the evolution of sulfide in porewaters in these waters. We initially hypothesized that porewater sulfide production would be limited by both sulfate and the availability of total organic carbon (TOC) to sulfate-reducing bacteria. The initial model also considered that the availability of porewater iron (Fe) was constraining on porewater sulfide, and that the supply of dissolved Fe in turn was a function of sediment TOC and sediment extractable Fe. The model thus comprised three independent or exogenous variables: water column sulfate, sediment TOC and sediment extractable Fe; and three dependent or endogenous variables: sediment total S, and porewater Fe and sulfide. One of the strengths of SEM is that, unlike traditional ordinary least squares regression, it allows for non-recursive (feedback) relationships such as that expected between porewater sulfide and Fe. SEM also serves as a framework for causal interpretation. The initial model pathways accordingly were based on expected and mechanistically supportable inter variable relationships (e.g., increasing water column sulfate concentrations lead to higher porewater sulfide levels through increased rates of sulfate reduction when controlling for other variable effects). A variety of *post hoc* model evaluation metrics universally supported the hypothesis that our original model (with slight modification) was a reasonably robust representation of the key processes governing porewater sulfide. This conclusion was further validated by considering several modifications to the model to include other variable effects (*e.g,* total phosphorus) – all of which resulted in inferior performance vis-à-vis the original model. Our understanding of the variables that control porewater sulfide afforded by the SEM in turn provided a framework for investigating how other multivariate models (e.g., linear and logistic regression) might be employed to calculate waterbody-specific sulfate concentrations that correspond to potentially protective porewater sulfide concentrations.

682 Use of a Weight-of-Evidence Approach to Assess Sulfide Effects on Wild Rice in Minnesota

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The State of Minnesota is presently working to derive a surface water quality standard for sulfate in waters for the protection of wild rice. Although wild rice is unaffected by relatively high levels of sulfate (up to 5000 mg/L), it does show sensitivity to sulfide at lower levels. In freshwater shallow lake systems, such as those inhabited by wild rice, elevated sulfate is believed to result in elevated sulfide concentrations in porewater due to geochemical processes. Therefore, one step in the development of a sulfate water column standard is the identification of porewater sulfide levels that have adverse impacts on wild rice. A number of studies have examined the relationship between wild rice and sulfide which include (1) detailed field surveys, (2) small scale hydroponics studies, and (3) mesocosm studies. A challenge in evaluating the data is that the calculation of effects levels such as the 10% effects level (EC_{10}) in each study depend greatly on seemingly mundane choices during data analysis. Some of the most surprising factors that influences the effects levels included (1) determining the baseline response in the control (EC_0), (2) characterizing the exposure concentrations in the treatments, (3) defining the upper and lower asymptotes of the dose response curve, (4) the unexpected influence of data transformations, and (5) evaluating experimental design. We examined these studies in detail and documented the various EC_{10} that can be calculated for each study. Furthermore, we attempted to combine these results using a weight of evidence evaluation to derive a sulfide level sufficiently protective of wild rice. This analysis provides a very powerful example of the difficulties in deriving aquatic criteria for many chemicals such as sulfate and sulfide. It does not address the wider questions of (1) is porewater sulfide controlled by surface water sulfate and (2) is sulfide limiting wild rice distributions or are other factors more important.

683 Sulfate release in the taconite mining region of northeastern Minnesota

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Sulfate reduction is linked to numerous biogeochemical processes that commonly occur in lake, stream, and wetland sediments under anoxic conditions, and its impact on ecosystem health is of considerable concern. It is thus important to understand the source of the sulfate and its pathways into the environment. Iron sulfide minerals, exposed as a result of mine processing, can oxidize and release sulfate that makes its way from mine pits, stockpiles, and tailings basins into surrounding watersheds through a combination of surface discharges and subsurface seepage. While both pathways are relevant during the active mining phase, the latter has the potential to contribute to downstream sulfate loading long after mine closure. In order to better characterize this seepage component across the Mesabi Iron Range of northeastern Minnesota, the Minnesota Department of Natural Resources sampled waters on and adjacent to mine properties, focusing on five taconite tailings basins. Geochemical and isotopic techniques quantitatively demonstrated that as sulfate is transported away from the basins with groundwater, concentrations increase over some intervals of its flow path due to oxidation of sulfide minerals, while concentrations decrease over other intervals due to sulfate reduction and precipitation of iron sulfide. While the relative additions and subtractions vary from site to site, it is clear that both processes should be accounted for to adequately represent current and potential future conditions. One of the basins was chosen for an additional pilot reactive transport modeling study, to simulate subsurface sulfate release from the basin to nearby surface and groundwater. Six representative cross section models were implemented using available hydrologic and geochemical observations as inputs and calibration targets. Combining these efforts improves our understanding of the sulfate source and the site-specific factors that influence sulfate loading from tailings basins, and with further development, may potentially be used to help evaluate future site management and closure scenarios.

684 Microbial Ecology and Activities of a Sulfate-Reducing Bioelectrochemical Reactor in Northern Minnesota

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In northeast Minnesota, high sulfate level in fresh water system is a topic of interest due to its potential adverse impacts to wild rice ecosystems. Sulfate may also contribute to methylmercury production and eutrophication in certain conditions. Increased interest has emerged for developing technologies to treat the high levels of sulfate in the circumneutral water. Biological sulfate reduction is a promising and economically viable plan for maintaining low levels of sulfate and sulfide, but its performance is highly variable. In this work, low electrical potential is applied to stimulate and sustain the process by continually supplying electron donor substrates to the sulfate reducing bacteria. Simultaneously, anodic iron dissolution from a stainless-steel electrode occurred and reacted with sulfide produced from biological sulfate reduction, to form iron sulfide. Sediment bioelectrochemical reactors were used to test the effect of low voltage on the efficacy of sulfate reduction and iron sulfide formation. Reactors contained creek sediment impacted with high strength sulfate (Second Creek, MN). Synthetic mine water with a sulfate concentration of 1000 ppm was treated within reactors operated at different voltages with control reactors (open circuit). The sulfur chemistry in the pore water of the reactors was assessed to determine microbial activity; this resulted in demonstrating active sulfate reduction occurred. Microbial community structure and relative abundance of different species associated with sulfate reduction in the reactors were examined. This study will result in a proof of concept application of electrical potential to enhance the performance of biological sulfate treatment in a controlled manner.

Microplastics in the Aquatic Environment – Fate and Effects

685 Removal of microplastic from three wastewater treatment plants in Charleston, SC

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Microplastics have been discovered in water, sediments, and biota in both freshwater and marine systems all over the world, and take many forms – from spheres to fragments or fibers of varying polymeric composition. It is important to identify sources of microplastics into the environment in order to create effective solutions for source reduction and limit potential risks to human and ecological health. Wastewater treatment plants (WWTPs) serve to collect and treat wastes that are known to include microplastic particles, fibers and microbeads. WWTPs are often cited as one of several potential sources of microplastics into the aquatic environment, and the few published studies available quantify concentrations in treated effluent water that vary over four orders of magnitude. This study aims to determine the loading, removal efficiency and type of microplastic through three conventional WWTPs in Charleston, SC that vary in treatment process, size, and service composition. This study is unique in that samples were collected several times over the course of a year. Influent, effluent and sludge samples from each WWTP were analyzed using 1) physical separation (size fractions >418 um, 178-418 um, and 178-60 um), 2) chemical digestion, and 3) counting by light microscopy. Preliminary results find 2 to 26 microplastics per L of treated effluent. Removal efficiency was greater than 92%, with high recovery of microplastic in sludge. Microplastics in treated effluent were predominately fibers, although in influent there was a larger contribution of particles including microbeads, indicating preferential removal of particles through treatment. Microplastic concentration was consistently lowest in the largest treatment plant that employs primary clarifiers. Sampling over time captured an effect of primary screening size as well: at one WWTP, the change from a 6 mm bar screen to a 3 mm perforated bar screen resulted in reduced microplastic concentration in effluent by a factor of 4. Pending results will continue to establish removal effectiveness over time and with changes in plant operations, and will use micro-FT-IR to identify predominate polymer types in wastewater treatment plant samples. Findings indicate that removal of microplastic by wastewater treatment plants is efficient, but can be improved by selection of primary treatment.

686 Tire Wear Particles: Occurrence, Fate, and Effects in the Charleston Harbor Estuary, South Carolina

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Microplastics (< 5 mm) are ubiquitous in the environment, and abundances as high as thousands of particles/m^2 are present on shorelines worldwide. A previous survey of Charleston Harbor, SC reported an average of 591 ± 103 microplastic particles/m^2 in intertidal sediments, with black fragments suspected to be tire wear particles making up >90% of the particles at some sites. The objective of the present study was to further characterize the abundance and distribution of microplastics, in particular, tire wear particles, in an effort to identify sources in Charleston Harbor. As rivers are thought to be a contributor of non-point and point source microplastics, three major tributaries of Charleston Harbor—the Ashley River, Cooper River, and Wando River—were surveyed. Intertidal sediment (n=6), subtidal sediment (n=3), and sea surface microlayer (n=3) samples were collected from three sites (upstream, midstream, downstream) along each of the rivers and were analyzed for microplastics (63-500 µm). Intertidal sediment abundance ranged from 0-680 particles/m^2. Subtidal sediment abundance ranged from 3-2916 particles/kg wet weight. Sea surface microlayer abundance ranged from 3-36 particles/L. Microplastic abundance in intertidal sediments, subtidal sediments, and the sea surface microlayer did not differ significantly among upstream, midstream, or downstream sites. When the data were pooled, microplastic abundance in intertidal sediments and the sea surface microlayer differed significantly among rivers ($p< 0.0001$ (intertidal), $p=0.003$ (sea surface microlayer)), with the Cooper River containing the fewest microplastics (63 ± 13 particles/m^2, 5 ± 1 particles/L). Microplastic abundance in subtidal sediments did not differ significantly among rivers. Blue microplastic fibers and black tire wear particles were the two most abundant types of microplastics observed, constituting 26% and 28%, respectively, of total microplastics collected. Furthermore, because the Cooper River contained the fewest microplastics but the greatest number of point source discharges, these results suggest that non-point sources are a significant contributor of microplastics in Charleston Harbor. Data regarding the acute and chronic toxicity of tire wear particles, including their effect on immune function, in grass shrimp (*Palaemonetes pugio*) will be presented. These results are the first to report on the occurrence, fate, and effects of tire wear particles in a southeastern U.S. estuary.

687 From Rivers to Lake - Variability in the Vertical Distribution of Microplastics in the Water Column and Surficial Sediment in Milwaukee, WI

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Microplastics–plastic particles less than 5mm in diameter–have become a contaminant of concern in freshwater systems. Potential *pathways* which introduce various microplastic sources to freshwater systems include, but are not limited to, the breakdown of larger plastic litter in *urban runoff* (e.g., Styrofoam, plastic bags, bottles, wrappers, cigarette butts) and *wastewater treatment plant effluent* (e.g., synthetic fibers from clothing and textiles, fragments of larger debris, microbeads from personal care

products). To date, previous research on microplastics focused on the surface of a water-body to quantify occurrence and abundance. Little, if any, research has been done to characterize microplastic prevalence at different depths within the water column. In 2016, the U.S. Geological Survey, Milwaukee Metropolitan Sewage District, and State University of New York at Fredonia collaborated to characterize the vertical distribution of microplastics in the water column across ten sampling locations ranging from rivers to nearshore Lake Michigan. Surface-water samples from the top 15-30cm were collected using a 3 foot by 1.3 foot 0.335 mm mesh neuston net. One surface sediment (top 20cm) sample was composited from multiple subsamples at nine of the sampling locations. At six of the ten sampling locations additional water samples were collected at certain depth intervals within the water column using a 6 inch diameter 0.335 mm mesh net. Mean total particle concentration in water samples from all sites and depths was 1.78 particles/m^3, with a maximum concentration of 19.1 particles/m^3, and a median of 0.85 particles/m^3. Site-specific mean concentrations ranged from 0.5 to 11.6 particles/m^3. Fibers/lines were the dominant particle type in water samples, making up 80 percent of the 15,545 microplastic particles collected. A comparison of surface and depth-weighted microplastic concentrations suggest water velocity and particle buoyancy could be potential factors governing estimates of the distribution of microplastic concentrations within the water column. At upstream river sampling locations with higher unidirectional water velocities, particle buoyancy appears less important, compared to the more tranquil downstream harbor and lake locations with less unidirectional water velocities. Our results suggest inclusion of multiple sampling points within the water column improved accuracy of overall microplastic assessment compared to sampling only the surface of the water body.

688 Understanding impacts of microplastics on Eastern oysters

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Microplastics, or plastics smaller than 5mm, present a real, but poorly understood threat to the health and viability of marine ecosystems and the seafood industry. Microplastics can cause direct damage to marine biota, absorb hazardous chemical contaminants, and bio-accumulate in benthic invertebrates and fish destined for human consumption. Microplastics are prevalent around the world and research at the Dauphin Island Sea Lab has demonstrated they are also found in Gulf of Mexico waters and along the shoreline, but the potential impacts on key marine resources remain unknown. Microplastics can be found in everything from the water we drink to the food we eat, however, we do not yet know if this has a negative or negligible impact on marine organisms, marine food webs, and ultimately human consumption of seafood. To examine this juvenile eastern oysters, *Crassostrea virginica*, were used to investigate the uptake, fate and chronic effects of microplastic ingestion. Over the course of three months oysters were exposed to a variety of single and combined treatments of polyethylene (10-50 µm), polystyrene (20 µm), and ethinyl estradiol (EE2). During this time their growth, respiration, filtration, and plastic loads were measured to determine if microplastics and associated toxins were having any effect on the oysters. Lastly, we examined oyster tissues by visual and chemical means to determine the residence time of various plastics and residues of EE2 desorbed from plastics and taken up in their tissues. In addition to this experiment, we also examined oysters from the same spawning group but raised in situ over the course of nine months for comparison. Using growth metrics of tissue and shell weight a condition index was calculated monthly to determine the 'health' of the oysters, an average, healthy oyster has a condition index of 10. In this experiment condition values ranged from a minimum of 7.03 in the oysters that were exposed to polyethylene, polystyrene, and EE2 to 23.06 in the oysters raised in situ. With these data we hope to determine the impacts of microplastics as well as evaluate the potential use of oysters as a bioindicator of microplastics contamination in local bays and coastal zones.

689 Incorporation of titania and polystyrene nanoparticles into marine snow: Implications for ingestion and bioaccumulation in bivalve shellfish

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In aquatic environments, most particles are not monodispersed but form homo-agglomerations and hetero-aggregations (e.g., marine snow) that are 10s to 100s of micrometers in size. Understanding the tendency of different nanoparticles (NP) to associate with hetero-aggregations, and the resulting effects on capture and ingestion, is important for a knowledge of NP bioaccumulation. In suspension-feeding bivalves, particle capture and ingestion is a direct route for the uptake of anthropogenic contaminants. These feeding processes are, however, constrained by functional mechanisms, and most bivalves capture particles Mytilus edulis) and eastern oysters (*Crassostrea virginica*). NP differed in composition and size, and included: 1) Titan (surface-coated, 93% rutile TiO_2, 6% Al_2O_3, 1% glycerin; 86 nm); 2) P25 (76% anatase TiO_2, 24% rutile TiO_2; 28 nm); 3) Nanopolystyrene (50 nm); and 4) Nanopolystyrene (100 nm). NP were delivered to bivalves either incorporated in marine snow (laboratory generated, 3 days) or freely suspended at concentrations of 1 mg/L. The lowest incorporation efficiency was found for Nanopolystyrene (50 nm = ca. 16%, 100 nm = ca. 58%), whereas titania was incorporated at higher efficiencies (Titan = ca. 70%, P25 = ca. 97%). Pumping rates of bivalves in each treatment were similar, and except when delivered 100-nm Nanopolystyrene, the percentage of NP ingested per hour by both bivalve species was independent of form of delivery (NP in marine snow = freely suspended). For 100-nm Nanopolystyrene, both species of bivalves ingested significantly more NP when they were incorporated in marine snow, although the amount ingested as a proportion of the total amount delivered was low (< 1%). Results demonstrate that for some NP, incorporation into marine snow can substantially increase ingestion. For other NP types, formation of homo-agglomerations produce masses large enough to be captured and ingested by bivalves.

690 Synthetic polymer contamination in two American consumer products and global drinking water

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Plastic pollution has been well documented in natural environments, including the open waters and sediments within lakes and rivers, the open ocean and even the air, but less attention has been paid to synthetic polymers in human consumables. This study explores anthropogenic debris in twelve brands of table salt, twelve brands of beer, and 159 samples of globally sourced tap water. Synthetic polymers were found in each brand of salt and beer, the majority of them fibers, 99.3% and 98.3% respectively. After adjusting for particles found in lab blanks for both salt and beer, the average number of particles found in table salt was 212 n/Kg with a range of 46 n/Kg to 806 n/Kg and the average number of particles found in beer was 4.04 n/L with a range of 0 n/L to 14.3 n/L. Of the tap water samples analyzed, 83% were found to contain plastic particles. The majority of these particles were also fibers (99.7%) between 0.1 - 5 mm in length. The range was 0 to 57 n/L, with an overall mean of 4.34 n/L.

691 Assessing Microplastics in the Mississippi River Watershed and Their Discharge to the Gulf of Mexico

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The ubiquitous presence of plastic debris in the ocean is recognized by the public and scientific communities. Most of this debris is considered microplastic (< 5 mm diameter) which due to their small size were not widely recognized until the last decade. While there are estimates for the total amount of plastic debris in the ocean, loading from many of the major sources have not been quantified. The Mississippi River, with its headwaters nearby in Minnesota, is likely one of the largest sources of oceanic plastic debris in the U.S., and globally. This research funded by the NOAA Marine Debris Program will quantify and characterize (size, shape, resin type) microplastics within the main stem of the Mississippi River including major cities such as St. Louis and New Orleans as well as contributions from major tributaries (Missouri, Illinois and Ohio Rivers). Sampling is ongoing, but the conclusive data will enable estimates of total microplastic loads and concentrations, spatial and temporal trends, land-use effects and total discharge of microplastics to the Gulf of Mexico. This research will produce a baseline that can be utilized in future research relating to the fate and effects of microplastics in aquatic environments as well as aid federal and local policy makers in creating and assessing mitigation strategies to improve water quality.

692 Needed improvements in microplastic hazard research

K.A. Connors, S.D. Dyer, S.E. Belanger, The Procter & Gamble Company / Environmental Stewardship and Sustainability Organization

An exponential growth in scientific publications regarding the environmental fate and effects of microplastics and an increase in regulatory attention is ongoing. Despite these efforts, the environmental hazard of these particles is still relatively unknown. To evaluate the hazard of microplastics within a risk assessment context, we need a way to evaluate the quality of experimental studies. A literature review was performed to assess the state of the science. Seventy-one manuscripts were identified that attempted to identify and quantify microplastic hazard concentrations. We reviewed of the focus, scope and the methods of this work to understand how this research may assist or distract from the ability of environmental risk assessors to evaluate microplastics. Here, we provide guidance to improve the reliability and relevance of ecotoxicological studies for decision-making. Overall, we found inconsistent particle preparation techniques, incomplete reporting of particle attributes (e.g. density, charge) and limited discussion on agglomeration/aggregation as major limitations within the microplastics literature. Significant data gaps in particle size, polymer type, and tested species were identified. Nine areas of for improvement were identified and will be discussed including: environmental relevance of test concentrations; provision of sufficient detail for converting particle concentrations; thorough particle description and characterization; reporting particle preparation techniques and suspension stability; analytical verification of test concentrations; consideration of environmental relevance of particle size; inclusion of appropriate controls (chemical and biological); consideration of endpoint applicability to environmental risk assessment; reporting findings accurately, without conjecture beyond experimental limits.

Environmental Data Mining – Doing Research With No Money

693 Opportunities and Challenges of Environmental Data Mining

L.A. Rodenburg, Rutgers University / Environmental Sciences

This presentation will examine the lessons learned from a decade of environmental data mining. Successful data mining depends on knowing where to find the environmental data. While, data sources are proliferating, finding the data is still harder than it should be. For example, filing freedom of information requests only helps if you know who has the data. When data are being requested, it always is better for data to be given willingly rather than grudgingly. It is also important to get the data in the right format. Poor data management is currently a huge obstacle to data mining; too many cooks in the kitchen lead to incompatible data sets. When searching you should know where to find the ancillary data, which will help you understand the environmental data. This issue creates “new life” for librarians. It is also important to understand net neutrality when you are searching for esoteric things. In addition, the presentation will ask if you have the right tools to analyze the data: Are they free/publicly available? Are they reliable/publishable/defensible? Are they appropriate for your purpose? Do you have the expertise to run these models/tools, such as statistics, GIS, etc.? Having the expertise to understand the output of the models will result in comprehensive knowledge of things like fate and transport.

694 Source Apportionment of Polychlorinated Biphenyls in District of Columbia Wastewater

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Polychlorinated biphenyls (PCBs) are persistent and toxic pollutants which bioaccumulate in the environment. Exposure to PCBs can cause a multitude of health issues including neurological disorder, reproductive toxicity and deformity, endocrine disruption, and cancer. Positive matrix factorization (PMF) has been used to examine a data set in order to apportion sources of PCBs in wastewater effluent in the District of Columbia. The fate of PCBs in wastewater is essential to evaluate the feasibility of wastewater treatment processes and the environmental risk. A data set containing 64 chromatographic peaks representing 94 PCB congeners measured in 89 whole water samples was analyzed using high resolution mass spectrometry (EPA method 1668 revision A). PMF resolved four factors. Three factors represent relatively unweathered Aroclors, specifically 1242, 1254, and 1260. The last resolved factor represents advanced dechlorination of PCBs. PCBs could be dissolved in wastewater effluent or sorbed onto particles. PCBs may undergo dechlorination to less toxic congeners in combined sewer systems before reaching the wastewater treatment facility. Once within the treatment facility, the compounds may further undergo volatilization during aeration as well as sorption and sedimentation in the sedimentation basins. In addition, PCBs may further undergo biodegradation in the treatment facility. Such biological, physical and chemical processes may assist in rendering these compounds less toxic as well as reducing the exposure to the public. Future research is aimed at identifying where most dechlorinating is occurring in the treatment system.

695 Atmospheric PAHs in Minnesota: Spatial Comparison with U.S. Long-Term Monitoring Sites, Source Characterization and Wind Influence

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Ambient atmospheric total (vapor + particle) PAH concentrations measured at urban and rural settings in Minnesota and other long-term monitoring sites around the Contiguous United States during 2013 – 2015 are reported. Average Σ PAH concentrations measured in Minnesota were not statically different than concentrations observed at other similarly situated long-term monitoring sites (urban sites $P = 0.0004$ and rural sites $P < 0.0001$). PAH source emissions for the regional background and urban and rural Minnesota sites were characterized using a multivariate discriminant function analysis using PAH profiles and previously reported PAH diagnostic ratios. The regional background PAH signature was characterized by higher coal and wood combustion and gasoline vehicle emission sources relative to the Minnesota urban site. The Minnesota urban site, in contrast, was characterized by higher diesel vehicle emission sources relative to the regional background PAH signature. The Minnesota rural PAH signature showed similarities with the regional background and Minnesota urban PAH signatures, but aligned more closely with the urban PAH signature suggesting the site may by influenced by nearby urbanization. To further investigate this influence, fractional wind direction data representing four vectors with varying population densities were regressed with total ∑ PAH concentrations. Winds predominantly from the sparsely populated north and the more densely populated west regions were significantly associated with lower ($P = 0.030$ and $R^2 = 0.098$) and higher ($P = 0.003$ and $R^2 = 0.178$) total ∑ PAH concentrations respectively. Similar relationships with the sparsely populated east and the more densely populated south regions, however, were not significant. These mixed findings underscore the need for continued research on PAH emission sources in Minnesota.

696 Predicting arsenic in drinking water wells in glacial aquifers in western and central Minnesota, USA

M.L. Erickson, S. Elliott, C. Christenson, USGS / Minnesota Water Science Center

Arsenic is a naturally-occurring contaminant adversely affecting drinking water quality sourced from groundwater in geologically diverse aquifers in Asia, Europe, Africa, as well as North and South America. Because arsenic is associated with occurrences of cancer, the World Health Organization and the U.S. Environmental Protection Agency (EPA) have established a drinking water standard of 10 µg/L. In Minnesota has an estimated 250,000 domestic water well users with drinking water arsenic concentrations above 10 µ/L (elevated arsenic). However, groundwater arsenic concentrations vary considerably over short distances and regionally across the state. Elevated groundwater arsenic is more prevalent from northwestern to south central Minnesota. Approximately 40% of available arsenic data for groundwater in western and central regions of Minnesota exceed the drinking water standard. A Boosted Regression Tree (BRT) model was developed to predict the probability of elevated arsenic in groundwater at typical depths used for drinking water supply in glacial aquifers in western and central Minnesota. Predictive factors used to build the BRT model were obtained from a variety of existing data sets and consisted of about 75 different factors such as well construction, glacial aquifer, and surficial (e.g. soil texture, soil chemistry, land use) characteristics. The final BRT model predicted the probabilities of elevated arsenic in groundwater with about 65% accuracy. Predictive factors determined to be important for predicted probabilities included clay gap (distance from top of screen to overlying confining unit), nearest major river (a proxy for hydrological position in the landscape), horizontal hydraulic conductivity, and distance to the top of the bedrock from the bottom of the well. For example, smaller clay gaps were typically related to higher probability of elevated arsenic concentrations. This is the first successful application of BRT to model probabilities of elevated arsenic in a glacial aquifer system. The BRT model results were then used to generate maps illustrating probabilities of elevated arsenic across the modeled regions. The maps illustrate probabilities of elevated arsenic at the depth and with the construction characteristics typical of domestic drinking water well. Controllable variables, such as clay gap, were varied in separate maps to illustrate how the probability of elevated arsenic changes with changing well construction choices.

697 Identifying Environmentally Relevant Signals and Curve Classes in Tox21 qHTS Data

F. Melnikov, Yale University / Forestry and Environmental Studies Center for Green Chemistry and Engineering Molecular Design Research Network; J. Zimmerman, Yale University / Environmental Engineering; P. Anastas, Yale University / Chemistry

Animal bioassays are expensive, time consuming, and often suffer from limited relevance to human toxicity endpoints. To supplement conventional testing strategies, U.S. Tox21 program profiled 10K-compound library across a panel of multiplexed, stress-response assays. However, the assay results can be difficult to interpret and use in chemical assessment or design due to technology artifacts and non-monotonic concentration-response curves (CRCs). To better characterize the signal types observed in Tox21 library, we propose a CRC fitting algorithm for the multiplexed quantitative high throughput screening (qHTS) data that accounts for non-monotonic CRCs and interactions between stress response and cytotoxicity endpoints. The algorithm combined heuristic data-processing with statistically-robust CRC fitting procedure and provides standard curve inference parameters (AC_{50} and E_{max}), as well as weighted area under the curve (wAUC), point of departure (POD), and the associated uncertainty estimates. Finally, we identify curve classes based on the curve shapes and interactions between the stress response and cytotoxicity endpoint to better qualify the biological effects observed in the assay battery. The inference from the curve-fitting algorithm will be used to identify hazardous chemicals and design safer alternatives in accordance with the 4th principle of green chemistry.

698 Toward Integrative and Holistic Watershed-level Risk Assessment: Data Challenges and Insights

M. Cains, D. Henshel, D. Good, Indiana University / School of Public and Environmental Affairs; D. Wild, Indiana University / School of Informatics and Computing

Multi-stressor and multi-endpoint risk assessments require data from multiple sources. Data in multiscale systems are often collected at different scales within the system and in disparate units of measurement which need to be integrated across scales and units into a single risk assessment. Furthermore, ecosystems are complex systems with multiple levels of biological organization, interacting food webs, interacting habitat needs, and impacts. Stressors vary by concentration and magnitude of impact across an ecosystem. Data about ecosystems and their multifunctional components are often sparse, focused on one (or a few) species, are obtained at different times (e.g. years, seasons), aggregated over different time frames (e.g. hourly, monthly), and collected using disparate methods. Global efforts (e.g. One Health) to develop more inclusive and holistic risk assessment frameworks and methodologies emphasize the need to integrate data from dissimilar data sources with dissimilar units of analysis. Using the Charleston Harbor Watershed (i.e. Cooper River Basin) in South Carolina as a case study, this research illustrates the data challenges associated with the implementation of a holistic watershed-level risk assessment. The featured risk assessment emphasizes the integration of temporal data from diverse domains (e.g. ecology, human health, social studies, economic development, or public policy), across three counties (Charleston, Dorchester, and Berkeley) with heterogeneous land use, and requires data curation from a variety of private, local, state, and federal sources. One of the challenges of integrating multiple domains is the need to reconcile dissimilar assessment metrics and measurement metrics. This challenge is further amplified by differing scales of spatial, temporal, and organizational units for each domain. We will discuss approaches to use in combining such dissimilar data, how to account for data uncertainty both statistically and visually, and how the data integration approaches inherent in risk assessment frameworks can influence the assessment outcome.

699 Introduction to ChemTHEATRE: Open data leads to a new era for the risk evaluation and communication

K. Nakayama, Ehime University / Center for Marine Environmental Studies; T. Isobe, National Institute for Environmental Studies / Center for Environmental Health Sciences; S. Uno, Kagoshima University / Faculty of Fisheries; I.C. Handoh, Niigata University / College of Creative Studies and Institute of Science and Technology; N. Ohno, University of Hyogo / Graduate School of Simulation Studies; D. Ueno, Saga University / Faculty of Agriculture; T. Kunisue, Ehime University / Center for Marine Environmental Studies

There is a general trend toward the growing importance of open data worldwide. It appears to be essential that development of scientific data repositories be accelerated. In the field of environmental chemistry and ecotoxicology, a huge number of monitoring data on chemicals in various environmental and biological specimens have been reported in scientific journals. However, comprehensive, public repositories to store such valuable data set of the chemicals do not exist; researchers are forced to spend lots of time and cost in collecting and utilizing the published data, when modelling environmental behavior and fate of, and performing the risk assessment for, the chemicals of interest. Therefore, it is desirable that various stakeholders in the field should work together to improve and promote secondary use of the data. To this end, we have created a platform to register and visualize the monitoring data of environmental contaminants, named 'ChemTHEATRE' (Chemicals in the THEATRE: Tractable and Heuristic E-Archive for Traceability and Responsible-care Engagement).

To date, data described in more than 30 publications have been registered on the platform. Users can find e-archived chemical concentration data in the environmental and biological specimens each with associated metadata such as sampling date and location, species, and biometrics, in addition to the detailed description of experimental methods. Bridging ChemTHEATRE to other databases storing chemical property and/or hazard/toxicity information provides us high accurate and transparent assessment of ecological risk of chemicals. Much effort is currently being devoted to visualizing e-archived data sets, and enhancing available data-model interfaces to simulate global dynamics of chemical pollution, with Finely-Advanced Transboundary Environmental model (FATE), and to promote a series of integrated exposure and effects analyses. It is thus expected that ChemTHEATRE will be not only a dedicated follow-up and forecasting tool of international regulations on pollution control in the light of traceability and responsible-care engagement of chemicals, but also a 'communication theatre' where a variety of stakeholders can improve their risk literacies and develop new projects through open data access.

Great Lakes Restoration Initiative – Occurrence and Effects of Contaminants of Emerging Concern

MP001 Derivation of Surface Water Screening Values for Hazard Scoring of Emerging Contaminant Impacts in Fish

D. Gefell, J. Banda, US Fish and Wildlife Service; S. Choy, US Fish and Wildlife Service / Ecological Services; S.E. Hummel, US Fish and Wildlife Service; B.C. Jorgenson, Pacific EcoRisk; J. Moore, US Fish and Wildlife Service / Interior; A. Roe, A. Secord, W. Tucker, L. Williams, US Fish and Wildlife Service

Ecological hazard assessments utilize ecotoxicological screening values (SVs) to assess efficiently the potential for adverse impacts to aquatic biota from exposure to environmental concentrations of chemicals. Surface water SVs are well-developed for legacy chemical hazard assessments in aquatic systems, but in the U.S., SVs for contaminants of emerging concern (CECs) are lacking. The U.S. Fish and Wildlife Service (USFWS) derived surface water screening values for a diverse set of 14 CECs to score relative hazard to freshwater fish due to direct uptake of dissolved CECs from water. The hazard scores may be used to rank relative hazard between sampling sites, between CECs, and between sampling events at specific sampling sites. We describe the underlying ecotoxicological database and derivation methods for the development of a flexible SV toolbox for hazard assessment in freshwater fish, which includes: *SV Point Estimate Distributions* for potential probabilistic hazard assessment applications, *Effect-Specific SVs* for custom hazard assessments, and CEC-specific *Final SVs* for classic hazard assessment applications. We present both upper bound and lower bound chronic SVs for both population and individual fish levels of ecological organization. Attributes of the USFWS SVs are compared and contrasted against existing chemical SVs for surface water hazard assessments. Appropriate applications of these SVs are intended to provide actionable information to natural resource managers, waste stream managers, and environmental agencies to facilitate decisions concerning further research and management actions.

MP002 Derivation of Empirical Uncertainty Factors for Deriving Surface Water Screening Values for Emerging Contaminant Impacts in Freshwater Fish

D. Gefell, J. Banda, US Fish and Wildlife Service; S. Choy, US Fish and Wildlife Service / Ecological Services; S.E. Hummel, US Fish and Wildlife Service; B.C. Jorgenson, Pacific EcoRisk; J. Moore, US Fish and Wildlife Service / Interior; A. Roe, US Fish and Widlife Service; A. Secord, W. Tucker, L. Williams, US Fish and Wildlife Service

The diversity of potential CEC exposure scenarios in aquatic species is usually large and unknown, due to the existence of numerous potential receptors, chemical contaminants, and environmental exposure conditions. In contrast to the complexity of real aquatic systems, reliable lab-based ecotoxicological information is limited to effects from a very small fraction of possible chemical contaminants, in relatively few receptors, and within a limited, controlled range of non-contaminant stressor conditions. The use of laboratory-based data to assess chemical impacts to ecological receptors in environmental exposure scenarios therefore requires simplifying assumptions. When attempting extrapolations of laboratory effect concentrations to the potential for effects in natural systems, uncertainty is often organized into categories, and uncertainty factors (UFs) are developed for each category. Based on an exhaustive literature compilation, the U.S. Fish and Wildlife Service developed empirical UFs for the Inter-species, Intra-species, and Chemical Complexity sources of uncertainty for CEC hazard assessments in freshwater fish. We present the database and detailed methods for deriving empirical uncertainty factors for both upper bound and lower bound screening values (SVs), for applications involving potential effects at the population and individual levels of ecological organization. Final empirical UF values are provided.

MP003 Pollutant Stress in the Maumee River: Impacted Physiology and Reproduction in Fathead Minnows (Pimephales promelas) and Sunfish (Lepomis spp.)

N. Cipoletti, St. Cloud State University / Aquatic Toxicity Laboratory; H.L. Schoenfuss, St. Cloud State University / Aquatic Toxicology Laboratory

Agricultural pollutants are an environmental health concern as precipitation can lead to runoff into aquatic ecosystems, resulting in stress for fish. The biological impacts of mixtures of agricultural pollutants, such as pesticides, herbicides, growth hormones, and livestock pharmaceuticals have yet to be studied. The objective of this field-based study was to assess the impact of agricultural pollutants on the physiology, reproduction, and population health of two fish species. The health of caged and resident sunfish was assessed in the Maumee River (Toledo, OH) as part of the Great Lakes Restoration Initiative. Laboratory cultured larval and adult fathead minnows were exposed for 21-days. Sunfish were analyzed for histology and hematological characteristics (VTG, glucose). Minnows were analyzed for alterations in hematological characteristics (VTG, glucose, 11-KT, E_2) and reproduction. VTG concentrations in male caged sunfish were significantly higher than in resident sunfish, likely due to greater energy stores in hatchery reared sunfish. Glucose concentrations between treatments varied significantly from upstream to downstream, possibly as the result of pollutant exposure. Biological indices including body condition factor, gonadosomatic index, and hepatosomatic index of resident sunfish also differed significantly across field sites. Fathead minnow fecundity was reduced in fish exposed to environmental samples from downstream, more urbanized sites. The results indicate that agricultural pollutants entering aquatic ecosystem have an impact on fish physiology and reproduction. Further research is underway to determine whether the observed physiological impacts have any effect at the population level.

MP004 A Screening Assessment of Trace Organic Contaminants in National Parks of the Midwestern United States

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Trace organic contaminants are ubiquitous in the environment. However, data gaps exist regarding the presence of these contaminants in national parks and the potential threats to wildlife. Monitoring efforts have been conducted in several Midwestern parks over the past decade to determine the extent of the presence of trace organic contaminants in national park surface waters and bald eagle (*Haliaeetus leucocephalus)* nestling (eaglet) plasma. Surface-water samples were collected during 2013-2017 from lakes and rivers of eight national park units representing a range of watershed influences including urban, forested, industrial, and agricultural. Water samples were analyzed for pharmaceuticals, personal care products, and pesticides. More chemicals were detected, and at higher concentrations, in the two more urban influenced parks. Atrazine and DEET were consistently detected at all parks, although relatively lower concentrations were observed at remote parks compared to those in more disturbed watersheds. Taking into account the number of chemicals analyzed, pesticides were detected at a higher rate than other chemicals. Surface-water concentrations rarely exceeded available human health-based and aquatic life screening values. In a separate effort, plasma samples were collected from eaglets during 2006-2015 within three Midwestern national parks. Plasma samples were analyzed for flame retardants, perfluorinated chemicals, and personal care products. Brominated flame retardants, bisphenol A, and perfluorinated chemicals were frequently detected in plasma collected in several parks. Similar to results seen in water samples, higher concentrations generally occurred in parks located in more disturbed watersheds. Results from both efforts are being screened against chemical bioactivity profiles published in the U.S. Environmental Protection Agency's ToxCast database to determine the threat these chemicals pose

to national park aquatic resources and wildlife. Preliminary results indicate that environmental concentrations of most chemicals are well below concentrations expected to elicit a toxicological response. However, the potential threat these chemicals pose to surface waters and wildlife may be exacerbated by exposure to mixtures of chemicals.

Neonicotinoid Insecticides – Potential Impacts on Non-target Organisms and Ecosystems

MP005 Environmental Fate and Toxicity of Neonicotinoid Insecticides

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Neonicotinoid insecticide are a popular class of systemic insecticides which have seen tremendous growth in use, due to their effectiveness at killing insects while demonstrating reduced toxicity to birds and mammals. They have recently come under scrutiny for their role in causing Colony Collapse Disorder in beehives. The purpose of this research was to determine the hydrolytic and photolytic reaction rates of several neonicotinoids, as well as the identify the products of the breakdown reactions. The toxicity of reaction products to non-target organisms was also determined. Hydrolytic reaction rates were determined by monitoring disappearance using high pressure liquid chromatography (HPLC). Rates were determined in a variety of matrices, including the presence of various metal ions and minerals. Photolysis experiments were conducted in a solar simulator, and samples were also analyzed by HPLC. At pH 10, hydrolysis half-lives were found to range from 10 days to 500+ days, but at environmental pH values, the half-lives were several years. Photolysis half-lives ranged from ~5 minutes to ~1.5 hours using simulated sunlight. Further research is being done to determine the toxicity of these breakdown reaction products to non-target organisms, and to identify the reaction products.

MP006 Modeling clothianidin surface water exposures in Canada

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Clothianidin is a neonicotinoid insecticide currently under registration review in Canada by the Pest Management Regulatory Agency (PMRA). Both monitoring and model-predicted exposure concentrations are important lines of evidence in risk assessments. In cases of minimal available monitoring data, modeling may be used to contextualize monitoring data and serve as the basis for risk characterization. The objective of this analysis is to present a refined exposure modeling approach that integrates the best available spatial datasets with current regulatory aquatic exposure models to estimate the likelihood and magnitude of clothianidin concentrations in potential aquatic invertebrate habitat. Modeled clothianidin concentrations were predicted using standard PMRA regulatory models with refined input assumptions to characterize regional differences in agriculture and aquatic habitat. Refined exposure modeling differed from screening-level analysis in model input parameterization and in the number of simulations used to develop a probability distribution of expected environmental concentrations (EECs). A large ensemble of 50-year simulations (realizations) was generated for specific crops and regions of interest. The inputs for each simulation that contributed to the probability distribution of EECs were derived from the likelihood of observed environmental conditions and application practices (e.g. water body size, percent of drainage area cropped, application timing) occurring within that region. This analysis uses model predictions to complement monitoring data and compares measured and modeled exposure distributions of clothianidin. A demonstration of how both tools can be used complimentarily in ecological risk assessment is the topic of a companion presentation.

MP007 Occurrence of neonicotinoid insecticides and fipronil in river waters in Saitama, Japan

N. Ohtsuka, K. Minomo, Center for Environmental Science in Saitama

Seven neonicotinoid insecticides (dinotefuran, clothianidin, imidacloprid, thiamethoxam, acetamiprid, thiacloprid, and nitenpyram) and a phenylpyrazole insecticide fipronil are widely used in the world. Recently, direct and indirect adverse effects of these insecticides to the ecosystem are concerned. Since environmental pollution by these insecticides is not well known in Japan, in this study, the concentrations of river waters were measured at 38 sites in 35 rivers in Saitama Prefecture in every season. Because neonicotinoid insecticides have been detected in human urine at high concentrations (μg/L level), we also investigated the concentrations of eight insecticides in influents and effluents from ten wastewater treatment plants (WWTP). At least one insecticide was detected at all sites except for one site located in the upstream mountainous area. Dinotefuran was the predominant insecticide in this study, followed by clothianidin, imidacloprid, and thiamethoxam. The highest concentration was observed for dinotefuran at 250 ng/L. The shipments of dinotefuran were the highest at 157.4 t in the neonicotinoid insecticides, followed by clothianidin (69.7 t) in Japan in 2013. The shipments of neonicotinoid insecticides can reflect the environmental distribution of these insecticides observed in this study. Moreover, we found seasonal variations of concentrations for the neonicotinoid insecticides; concentrations in summer were significantly higher than other seasons. This suggests that the insecticides can be more used in summer. Neonicotinoid insecticides and fipronil were detected in all effluent samples. The concentrations of imidacloprid and fipronil tended to be higher in the WWTP effluents than those in the river waters, whereas those of the other insecticides were comparable. Concentrations of the targets were similar between in receiving and effluent water in the WWTPs, indicating that neonicotinoid insecticides and fipronil were less removed and/or decomposed in the sewage treatment process such as activated sludge treatment. The results showed that not only use of neonicotinoid insecticides in the farmland, but also WWTP effluent is considered as a significant source of neonicotinoid insecticides and fipronil in the aquatic environment. This study was supported by JSPS KAKENHI Grant Number JP15K00573.

MP008 The use of polar organic chemical integrative samplers (POCIS) to assess the occurrence of neonicotinoid insecticides in surface waters

R.L. Dalton, University of Ottawa / Biology; S. Robinson, Environment and Climate Change Canada / Wildlife and Landscape Science Directorate

Global use of neonicotinoid insecticides on a variety of crop species is increasing. Concern has arisen regarding the potential effects of neonicotinoids on aquatic ecosystems due to their widespread use and moderately high solubility in water. Similar to other pesticides, the occurrence of neonicotinoids in surface waters is challenging to assess because concentrations are often highly variable across spatial and temporal scales. Traditional point-in-time estimates of concentrations (e.g. grab samples) do not integrate concentrations over time and this can result in a lack of understanding of actual exposures to aquatic biota. We used polar organic chemical integrative samplers (POCIS) to obtain time-weighted-average concentrations of neonicotinoids (acetamiprid, clothianidin, imidacloprid and thiamethoxam) in a large Canadian agricultural watershed in 2015 and 2016. POCIS were deployed in triplicate at eight stream and eight wetland sites surrounded by a range of agricultural land use. At least one neonicotinoid was detected in 75% and 88% of the field sites in 2015 and 2016 respectively. A laboratory calibration study was subsequently conducted to derive POCIS sampling rates for neonicotinoids to convert POCIS results (ng/POCIS) into water concentrations (ng/L). Grab sample and POCIS estimated concentrations of neonicotinoids in surface waters will be compared and the potential risk that neonicotinoids pose to aquatic biota discussed.

MP009 Chronic toxicity of neonicotinoids to aquatic invertebrates

M. Raby, University of Guelph / School of Environmental Sciences; D.G. Poirier, Ontario Ministry of the Environment / Laboratory Services Branch; P.K. Sibley, University of Guelph / School of Environmental Sciences

Neonicotinoids, a group of water soluble pesticides, are commonly applied as seed treatments to agricultural fields for the control of sucking insect pests. Transport to nearby waterways presents the potential for toxicity to non-target aquatic invertebrates, especially aquatic insects. Neonicotinoids are frequently detected in North American streams and ponds with mean concentrations in the ng to low µg L^{-1} range and may persist in sediments. Aquatic invertebrates therefore have the potential to be exposed to long-term (chronic), low-level neonicotinoid contamination. Our study investigated the effect of chronic neonicotinoid exposure to five species: *Chironomus dilutus* (midge), *Hyalella azteca* (scud), *Neocloeon triangulifer* (parthenogenic mayfly), and the cladocerans *Daphnia magna* and *Ceriodaphnia dubia*. For most species, six neonicotinoids were studied: imidacloprid, clothianidin, thiamethoxam, acetamiprid, thiacloprid, and dinotefuran. *H. azteca* was studied with only imidacloprid, clothianidin, and thiamethoxam, and *D. magna* with only imidacloprid and thiamethoxam. Exposures were semi-static and water-only in nature, and followed Environment Canada, USEPA, or published methods, with minor alterations discussed. Test durations followed species' life-cycle, and ranged from 7-d for *C. dubia*, to ≥56-d for *C. dilutus*. Data presented will include EC_{10}/EC_{50} estimates for chronic survival, growth, emergence (for *C. dilutus* and *N. triangulifer*), and reproduction endpoints. These data, along with literature values, are then used to generate long-term species sensitivity distributions (SSDs) for each neonicotinoid. The results of these SSDs are then compared to results of ongoing neonicotinoid monitoring projects in Ontario in a preliminary hazard assessment. This study is in partnership with the Ontario Ministry of the Environment and Climate Change, and will help to develop a comprehensive dataset from which to derive water quality criteria for the protection of aquatic life.

MP010 Derivation of an Aquatic Benchmark for Invertebrates Potentially Exposed to Imidacloprid Using the Results From Mesocosm and Field Studies

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Water quality benchmarks are developed by many jurisdictions worldwide with the general goal of identifying concentrations that protect aquatic communities. Imidacloprid is a widely-used neonicotinoid insecticide that is highly toxic to some classes of aquatic invertebrates including midges, mysids and mayflies. As a result, various jurisdictions have based their water quality benchmarks for imidacloprid on the results of laboratory toxicity tests conducted with single aquatic invertebrate species. Such tests do not account for environmental factors affecting bioavailability and toxicity, or species interactions and potential for recovery. Microcosm, mesocosm and field studies account for such factors and are available for aquatic invertebrate communities exposed to imidacloprid. We used the results of higher tier studies to derive a chronic water quality benchmark for imidacloprid. The first step was to screen the available higher tier studies for data quality and relevance. To this end, we developed a data evaluation rubric that determined which higher tier studies were acceptable, supplemental or unacceptable. Only acceptable studies were used in benchmark derivation. The chronic benchmark for imidacloprid was derived as follows: (1) for each taxon (family, subfamily or class depending on the study), we determined the most sensitive 21-day No Observed Effects Concentration (NOEC), (2) we fit the taxon NOECs to five distributions and determined the best-fit distribution, and (3) we determined the HC5 from the best-fit distribution. The higher tier chronic HC5 for imidacloprid is 1.01 µg/L, which is approximately two orders of magnitude higher than recently established chronic water quality benchmarks in Europe. The latter were based on the results of laboratory tests on single aquatic invertebrate species. Functional redundancy and the more realistic exposure conditions used in mesocosm and field studies likely explain this difference. Higher-tier studies should be used when available to derive water quality benchmarks because they offer a level of realism not attainable with standard laboratory toxicity tests.

MP011 A Refined Ecological Risk Assessment For Aquatic Invertebrates Exposed to Clothianidin in Canada

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Clothianidin is a neonicotinoid insecticide used in seed treatment and water dispersible granular formulations to control a variety of insect pests. Clothianidin is currently under registration review in Canada by the Pest Management Regulatory Agency (PMRA). In preparation for the registration review of clothianidin, we conducted a screening-level ecological risk assessment (SLERA) and refined ecological risk assessment to characterize risks to aquatic invertebrates from clothianidin use. The SLERA used highly conservative methods to assess potential risks to pelagic and benthic invertebrates from several worst-case clothianidin use patterns. Conservative risk quotients were above levels of concern (LOC) for acute exposures to clothianidin drift impacting the water column for freshwater invertebrates, and chronic exposure to clothianidin drift and runoff in the water column for freshwater and marine invertebrates. Risk to benthic invertebrates was deemed minimal based on limited LOC exceedance. Based on the results of the SLERA, the refined ecological risk assessment focussed on acute (drift only) and chronic risk of clothianidin use to aquatic invertebrates in Canadian surface waters. Previous Pesticide Registration Evaluation Decisions (PRVDs) conducted by PMRA (e.g. imidacloprid) have incorporated surface water monitoring data quantitatively in their exposure assessments. Therefore, clothianidin exposure distributions calculated using Canadian surface water monitoring data as well as refined exposure models were used in the refined exposure assessment. For the refined effects assessment, laboratory and mesocosm toxicity studies were evaluated for relevance and study quality. Data deemed acceptable or supplemental were used to derive species sensitivity distributions and/or dose-response curves. The refined assessment quantifies risk to aquatic invertebrates using probabilistic risk characterization approaches. Exposure distributions are integrated with effects distributions to create joint probability (or risk) curves that describe the probability of clothianidin concentrations exceeding different aquatic invertebrate effect levels. The results of mesocosm and field studies are also considered in the risk characterization as part of a weight-of-evidence approach.

MP012 An ecological risk assessment for thiamethoxam in North American surface waters: A weight of evidence approach

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The neonicotinoid insecticide thiamethoxam is widely used in agriculture across North America. It is detected more and more frequently in surface waters, in part due to increasing adoption by farmers to control crop pests. As a result, questions related to the ecological risk of thiamethoxam to aquatic ecosystems need to be addressed. To move our understanding forward, we used a weight of evidence approach to quantify the risk posed by thiamethoxam. All available toxicity data in the open literature were scored for their strength of experimental methods and the ecological relevance (survival, growth, development, and reproduction) of the responses reported. Numerical scores were assigned for strength and relevance. The means of the scores were then used to weigh the evidence for thiamethoxam contributing to ecologically significant responses. Our focus was on

Canada and the United States, where exposure data are currently greatest. The vast majority of measured concentrations are in the ng/L range, with µg/L observed under certain circumstances (e.g., downstream of greenhouses). There were also acute data of sufficient quality to create species sensitivity distributions (SSDs) for insects, generally seen as most sensitive, as well as less sensitive invertebrates (e.g., zooplankton). Together, the weight of evidence and SSDs revealed little likelihood of adverse outcomes for insects from acute exposure to thiamethoxam in terms of apical endpoints in most regions. There was effectively no risk observed for zooplankton, fish, and amphibians. Chronic exposures were less well characterized, and remain a point of significant uncertainty. We do know rapid removal (on the order of days) is expected for thiamethoxam barring continuous inputs. We recommend that chronic exposure in the environment be better characterized, and recognize the need for well conducted mesocosm studies to reduce uncertainty for pesticide risk assessors.

MP013 Imidacloprid persistence, mobility, and effect on soil quality and ecosystem function

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Systemic pesticide use appears beneficial to protect specific plants from economic and agricultural pests. Imidacloprid, a neonicotinoid pesticide is frequently used in agriculture and in the United States to protect hemlock trees from woolly adelgid infestations. Land managers rely on its systemic properties and persistence, however long-term studies investigating Imidacloprid effects on Appalachian soil ecosystems are limited. This study investigated three Imidacloprid applications to hemlock trees over a four-year period and compared residual concentrations in soil to measures of ecosystem function. These included soil respiration, microbial function, and macrofauna biodiversity and density. Soil samples were prepared using a QuEChERS method and concentrations quantified using liquid chromatography/mass spectrometry (LC/MS) with an MDL of 20 ng mL^{-1}. Microbial function was measured using Biolog EcoPlates™ and macrofauna biodiversity and density were determined by 24-hour transect sampling. Soil cores were collected along a transect from each tree at 0 m, 1 m, 5 m, and 10 m. Each core was also subsampled by depth. Sample locations were randomly selected and QA/QC protocol included three replicates, three duplicates, two spikes, and two standard additions per tree. Results indicate that Imidacloprid persists up to four years, is mobile, and can translocate into nearby plants. Average residual concentrations were highest near the application site for trees treated in 2013 (54.7 ppb, SD=36.5), 2016 (170.1 ppb, SD=163.9) and 2017 (86.7 ppb, SD=130.7) and were detected up to 10 m away from each tree (31.6 ppb, SD=12.2, 7.3 ppb, SD=6.6, and 40.8 ppb, SD=36.1 for trees treated in 2013, 2016, and 2017). Average concentrations decreased with depth for trees treated in 2013 (39.9 ppb, SD=31.5 at 0-10 cm to 35.8 ppb, SD=32.5 at 22-32 cm), 2016 (74.3 ppb, SD=130.2 at 0-10 cm to 31.4 ppb, SD=96.6 at 22-32 cm), and 2017 (70.8 ppb, SD=130.2 at 0-10 cm to 65.05 ppb, SD=71.7 at 22-32cm). Microbial function and macrofauna density and biodiversity were not significantly different between contaminated sites and the control. Concentrations in non-target plants were at levels potentially harmful to beneficial insects. Given the damage caused by woolly adelgid infestations, Imidacloprid is necessary in the protection of hemlock trees, but care should be taken where endemic, threatened, and endangered organisms reside.

MP014 Neonicotinoid Degradation by Microbes Isolated from Duckweed Roots

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Neonicotinoids are the most widely-used insecticide class in the world, and generate a variety of ecosystem effects. Understanding the ability of environmental microbes and plants to degrade neonicotinoids is crucial to minimizing the environmental impact of neonicotinoid usage. Recently published research from Canada demonstrated a correlation between vegetation type and neonicotinoid presence and concentration, causing us to hypothesize on the drivers behind these observed relationships. We collected duckweed and cattails from the environment and exposed these plants to imidacloprid (a widely-used neonicotinoid)-spiked liquid medium. The cattails did not alter imidacloprid aqueous phase concentration within seven days. The duckweed lowered imidacloprid concentration ~40% in ~three days. The same duckweed sterilized with bleach did not affect imidacloprid concentration, suggesting a non-plant uptake mechanism. Microbes isolated from the duckweed roots grew in a selective medium with imidacloprid as the sole nitrogen source, demonstrating that duckweed root-associated microbes are capable of degrading imidacloprid. Imidacloprid product levels were measured, and imidacloprid-degrading microbes were further tested for their ability to more broadly degrade other neonicotinoids.

MP015 Wetland sediment contamination by neonicotinoid insecticides: A study of Missouri land practices

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Neonicotinoids are among the most widely applied and fastest-growing class of insecticides commercially available for agricultural use (e.g., seed-treatments) in North America. Neonicotinoid physico-chemical properties (e.g., high water solubility, long half-lives in soils) enhance its environmental mobility and have led to detection of neonicotinoids in global surface waters including streams and wetlands of North America. Despite increased water sampling efforts, little is known about neonicotinoid concentrations occurring in wetland sediments. Thus, in 2016, we sampled water and sediment from 40 public wetlands under different management practices across Missouri during three sampling periods (pre-plant, post-plant and following autumn inundation). All samples were analyzed for the six most common neonicotinoid active ingredients. Pre-plant sediment most often contained clothianidin which was detected in 55% of samples (mean: 0.44 µg/Kg; max: 9.37 µg/Kg) with imidacloprid detected in 37% of samples (mean: 0.26 µg/Kg; max: 2.45 µg/Kg). Post-plant sediment samples frequently contained detectable residues of imidacloprid in 44% of samples (mean: 0.85 µg/Kg; max: 9.77 µg/Kg), followed by clothianidin in 31% of samples (mean: 0.0.39 µg/Kg; max: 7.85 µg/Kg). A subsample of fall sediment concentrations show similar patterns with clothianidin and imidacloprid detected in 43 and 32% of samples respectively. Neonicotinoids across all sampling periods were found to be an order of magnitude higher in the sediment than associated water, indicating cross-seasonal persistence in wetland sediments. We evaluated the relationship between watershed land-use, wetland management variables (e.g. crop planting), and concentration variability among wetlands, and found neonicotinoid concentrations increased proportionately with associated agricultural land in production. Results of this study will be useful in determining both potential routes and levels of neonicotinoid exposure to aquatic wetland invertebrates.

MP016 Neonicotinoid insecticides as drivers of trophic cascades: Using emerging aquatic invertebrates and tree swallows as model organisms

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Aerial insectivorous birds have experienced declines across much of North America during the past century. Habitat availability, land use change, and emerging and legacy contaminants have been implicated in population declines, and all, to varying degrees, may be contributors. Land conversion to mono-crop agriculture has further led to an increased need for pesticide application. The ubiquitous use of neonicotinoid seed dressings has shifted pest control efforts in the agricultural community from integrated pest management to a prophylactic approach. Widespread use combined with the highly water soluble nature of neonicotinoids has led to their detection in surface waters across North America and Europe, especially in areas of intensive agriculture such as the Midwestern United States. Non-target aquatic invertebrates are susceptible to neonicotinoid exposure as well as higher level organisms that rely on these invertebrates to cross from aquatic to terrestrial food-webs. To that end we established tree swallow (*Tachycineta bicolor*) nest boxes (n=100) at wetlands (n=20) across Missouri Conservation Areas. Study wetlands were paired within Conservation Areas to allow for the experimental application of neonicotinoid treated (thiamethoxam) corn seed on treatment wetlands, while planting the paired wetland with untreated seed. To evaluate the potential relationship between nesting and brood rearing success to neonicotinoid concentrations, we measured emergent insect biomass and recorded tree swallow nest volume and nestling growth rate, at approximately 35 nests at 14 wetlands during spring 2017. Average egg volume did not differ significantly between treated (1763.9mm^3) and reference (1758.7mm^3) wetlands. Preliminary results from a concurrent study of aquatic invertebrates suggest important tree swallow food sources, such as the invertebrates in family chironomidae, occur at lower abundances and smaller sizes at treated sites when compared to reference conditions. Reductions in important aquatic larvae may alter the amount and timing of emergent insect biomass transferring from aquatic to terrestrial food-webs. By evaluating the relationship among agricultural land practices, neonicotinoid concentrations, emergent insect biomass, and tree swallow nestling growth, we hope to elucidate the causal mechanisms associated with declining insectivorous bird populations in the presence of neonicotinoids.

MP017 Residual concentrations of neonicotinoids in honey, adults, pupae and larvae of honey bees from wild honeycombs and beekeeper honeycombs

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Neonicotinoid insecticides are widely used in Japan. Seven neonicotinoid insecticides such as Imidacloprid, Acetamiprid, Thiocloprid, Clothianidin, Dinotefuran, Thiamethoxam and Nitenpyram are popular. Their usage began from the beginning of 1990 and is increasing till 2008 in Japan. Recent annual total usage of seven insecticides is not increasing, approximately 400 tons per year in Japan. However, very little is known about their occurrence, their behaviors and their ecological risk in Japanese environment. Especially, there is little known about the exposure and ecological risk of neonicotinoids to wild bees in Japan though these neonicotinoid pesticides are considered to be one of the reasons for losses of bees in EU, Canada and the US. It should be noticeable that the residual levels of neonicotinoid pesticides in foods are much higher than those in EU and the US and that some news reported that losses of bees and honeycombs occurred recently in Japan. The aim of this research is to reveal ecological risk assessment of honeybees including loss risk of honeycombs in Japan. The exposure assessment is conducted by using field monitoring data in Japan. In order to collect data, we developed analytical methods to measure neonicotinoid concentrations in honey, honeybees, larvae, pupae, queen bee and pollen by ELISA kits. These samples were collected from beekeepers around in Japan. Wild honeycombs were also collected. The health condition of each bee colony could also be collected. The six neonicotinoids were detected in all samples including honey, pupae and larvae of honey bees. Especially, more than ten times higher concentrations were detected in some of honey bee samples than those reported by previous reports in Europe, Canada and America. All colonies where adult honeybees were exposed by high concentrations were evaluated as abnormal condition such as CCDs, massive fatalities and sacbrood disease.The concentrations in adult samples from wild honeycombs were also comparable to those from beekeepers. These results indicated that wild bee colonies and bee colonies kept by beekeepers were exposed by high concentration of neonicotinoids in Japan. The next step is ecological risk assessment for honey bees based on these data, EFSA guidelines and some of risk assessment tools such as BeeRex.

MP018 Quantifying Sources of Variability in Neonicotinoid Residue Data for Assessing Risks to Pollinators

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The U.S. Environmental Protection Agency's 2014 guidance for assessing pesticide risks to bees relies on higher-tier studies of residues in pollen and nectar to refine pesticide exposure estimates obtained from lower tier information (e.g., default values and models). These higher tier residue studies tend to be resource intensive due to the need to address spatial and temporal factors that influence pesticide residues in pollen and nectar. Time and resource considerations restrict the number of samples, crops and locations able to be studied. Given these resource constraints, questions remain on how to best optimize the design and number of residue studies for obtaining a robust dataset to refine estimates of pesticide exposure to bees. Factors to be optimized include the number of replicates in each sampling event, the number of sampling events over time, the number of sites per study, and the number of cropsto be assessed within and across crop groups. Using available field residue data for neonicotinoids, we conducted an analysis of variability in residue data to address these and other study design elements. Comparisons of the magnitude and variability of residues are made across neonicotinoid chemicals (imidacloprid, clothianidin, thiamethoxam and dinotefuran), within and between different crop groups, considering regional and soil texture gradients as well as the toxic metabolites for imidacloprid and thiamethoxam. Results of the neonicotinoid residue data analyses are presented in the context of optimizing field residue study designs for assessing pesticide risks to bees.

MP019 Development of a passerine model of chronic neonicotinoid toxicity using zebra finches

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Neonicotinoid insecticides are nicotinic acetycholine receptor (nAChR) agonists that are highly effective and commonly used in agriculture as seed coatings. While these compounds were developed to be insect nAChR specific, wild birds are at risk for toxicity at environmentally relevant levels, and reproductive, immune, and lethal effects have been documented in birds experimentally exposed to neonicotinoids. Therefore, we conducted a series of studies to examine the chronic toxicity of these compounds in a model passerine species, the zebra finch (*Taeniopygia guttata*). In the current study, birds were treated with 125, 250, 500, or 1,000 mg/kg (body weight) of clothianidin (CTD) on 1.5 g of millet seed offered to each bird. Within 2 hours we observed inactivity, failure to perch, fluffed feathers, respiratory difficulty, and, in some cases, inability to fly in individuals from all treatment groups. Clinical signs were generally increased at the higher treatment levels, although birds refused seeds coated with ≥ 500 mg/kg. Approximately 75% of the CTD coated seed was consumed by birds in the 125 mg/kg treatment group, representing a dose of approximately 100 mg/kg per bird. However, determining the concentration at which effects may occur was difficult because in the process of eating the seed the birds discarded the coated seed hull. By 24 hours after treatment, all of the birds returned to flight, perching, and normal behavior. At 14 days after treatment all birds were euthanized, histopathology was performed on liver tissue, and the brain acetylcholinesterase level was determined. Our work provides information on experimental exposure strategies for passerines in the laboratory in light of palatability and helps to set the range of CTD that might be used in a chronic toxicity trial in zebra finches.

MP020 Effects of Oral Neonicotinoid Exposure on Immune Function in Domestic Chickens (Gallus gallus domesticus)

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Evidence is accumulating that neonicotinoids can cause lethal and sublethal effects in vertebrates. Birds may be exposed to neonicotinoids by ingesting treated seeds, or via other means such as contact with contaminated soil or water. This active study is evaluating the effects imidacloprid (IMI) exposure on immune function in 6-12 week old white leghorn chickens (*Gallus gallus domesticus*). At least six doses of IMI will be evaluated, as well as a vehicle control group (Veh) and a positive control group using corticosterone (n=10 per treatment group). Current IMI doses include 0.037% (IMI 1), 0.33% (IMI 2), 3.3% (IMI 3), 10% (IMI 4), and 15% (IMI 5) of the reported LD_{50} in avian species (104.1 mg/kg). Birds are exposed to imidacloprid via gavage once daily for seven consecutive days. The following immune function assays are performed over the 21 day study: phytohemagglutinin-A (PHA) response test, sheep red blood cell (SRBC) antibody agglutination and hemolysis titers, and a delayed type hypersensitivity test. A microbicidal assay is being evaluated to measure the ability of plasma to kill *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* on various days during the study. Additional variables being evaluated include heterophil to lymphocyte ratios, clinical signs, and necropsy results. Preliminary data suggests there are no significant differences in the average PHA response, DTH response, SRBC agglutination titer and SRBC hemolysis titer between the Veh and IMI 1-4 groups. Temporary neurologic signs were observed in 100% of IMI 4 birds, 50% of IMI 3 birds, and 1% of IMI 2 birds. Neurologic signs ranged from mild depression to moderate sedation with increased respiratory effort, ataxia and whole body tremors. The moderate sedation would likely affect the bird's ability to survive in the wild. This study provides evidence that low to moderate doses of IMI may impair avian survival due to neurologic signs, but may not be immunotoxic. Additional results from this study will evaluate clinical signs and immune function at higher IMI doses in order to estimate quantile effects concentrations.

21st Century Approaches for Capturing Diversity in Species Sensitivity to Chemicals

MP021 Differences in ligand binding domain-mediated transcriptional activation between human and teleost fish estrogen receptors

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Xenoestrogens are a class of endocrine disrupting compounds (EDC) which act as estrogen mimics and can potentially cause reproductive dysfunction. Xenoestrogens mediate their effects by binding to estrogen receptors (ER), causing changes in transcriptional activation. These compounds can be found in the aquatic environment, where they are deposited as a result of land run-off and water treatment effluents, making their effects on fish populations a concern. Chemical screening programs are in place to detect potential compounds with estrogenic properties. However, the in vitro screening assays currently used for xenoestrogen detection are based on mammalian physiology, which differs drastically from those of teleost fishes. In particular, there are distinct differences between mammalian and fish ERs, including the amino acid sequences, number of subtypes, and subtype functions. Therefore, current assays may not accurately indicate the potential of chemicals to act as xenoestrogens in fish. In this study, we investigate the differences in xenoestrogen-mediated transcriptional activation between human and fish ER subtypes. In particular, this study focuses on the ER ligand-binding domain (LBD), which is known to confer the majority of substrate specificity in the response to estrogenic compounds. We utilize transactivation assays to compare ER LBD sensitivities between human and teleost ER subtypes. The hinge (D), LBD (E), and N-terminus (F) region of each ER subtype from human (hERα-def and hERβ-def), zebrafish (zERα-def, zERβ1-def, and zERβ2-def), and rainbow trout (rtERα-def, rtERα2-def, rtERβ1-def, and rtERβ2-def) are expressed as fusion proteins with a GAL4 DNA binding domain. GAL4-ERdef fusion proteins are used in transactivation assays, conducted in HEK293 cells, to compare the ER LBD-mediated response to a selection of natural and synthetic estrogenic compounds. Chemicals are ranked by the estradiol equivalency quotient for each receptor subtype to determine if human based in vitro assays provide similar answers to fish based in vitro assays with regards to xenoestrogen risk. This study evaluates the need for more targeted in vitro assays when assessing potential risk of EDCs to aquatic organisms.

MP022 Estimating Species Sensitivity Distributions (SSDs) Using Quantitative Structure-Activity Relationships (QSARs) for Organic Chemicals

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Species Sensitivity Distributions (SSDs) serve to estimate the proportion of species that may be affected by different concentrations of chemicals in the environment. Therefore, SSDs can be used to assess the ecotoxicological impacts of chemicals. However, due to the limited amount of measured acute ecotoxicity data for organic chemicals, SSDs cannot be constructed for many chemicals. Since Quantitative Structure–Activity Relationship (QSAR) models have been successfully applied to predict the toxicity of organic chemicals, they may be used to construct SSDs when experimental data is not available. Here we present an approach for constructing SSDs for organic chemicals based on QSARs. We first collected QSARs already developed for the Lethal Concentration (LC50) of *Pimephales Promelasand* and *Daphnia Magna*. We then developed LC50-based QSARs for several other species, including but not limited to: *Oncorhynchus Mykiss*, *Lepomis Macrochirus* and *Americamysis Bahia*. To develop the QSARs, experimental LC50 data was collected from databases such as ECOTOX and combined with additional literature data. The structural descriptors of organic chemicals were calculated using software Rdkit. A filter-based method and linear regression models were used to select significant chemical structure descriptors. The QSARs were developed with the state-of-art machine learning models such as Artificial Neural Network (ANN) and Random Forest. The Applicable Domain (AD) of each developed QSAR was characterized. The QSARs (from literature and newly developed) were used to create SSDs for a broad range of chemicals. We also estimated the uncertainty range of each SSD. The ecotoxicological impacts of the chemicals, even without experimental data, can be better understood using the QSAR-SSD framework in this study.

MP023 A Review of Cetacean Cell Culture in the Field of Toxicology: Methodologies, Applications, and Future Directions

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Whales and dolphins (cetaceans) are vulnerable to toxicants due to their often high trophic level positioning. Some species are in decline or in danger of becoming extinct. Over fishing, disease, ship strikes, and hunting are commonly associated with population declines, however the impact of xenobiotics is less understood. Toxicants found in cetacean tissues have been associated with cancer, reduced immunity, and reduced fecundity. Lethal toxicological investigations are not possible due to legal and ethical considerations regarding the species. Cell culture is a minimally invasive technique that is well suited to cetacean research. We conducted a literature review to investigate the scope of cetacean cell culture research and identify knowledge gaps. We then focused on the field of toxicology to better understand the applications used and identify knowledge gaps relating to xenobiotics in marine mammals. Toxicological research represented 57% (N-22) of all publications involving cetacean cell culture (N=38). These publications were from 1995-2013 and spanned the following areas of toxicological research: cytotoxicity, genotoxicity, induction studies, cytopathology, and biomarker studies. Nine species of cetaceans were investigated. The bottlenose dolphin (*Tursiops truncatus*) accounted for 36% (N=9) of species, followed by the striped dolphin (*Stenella coeruleoalba*) 12% (N=3), and the Atlantic spotted dolphin (*Stenella frontalis*) 12% (N=3). Endangered species included the North Atlantic right whale (*Eubalaena* glacialis) and fin whale (*Balaenoptera physalus*) and accounted for 16% (N=4) of all species included in the literature. Tissues included skin biopsies obtained from free swimming or captive animals and harvested organs (lung, kidney, and testis) from stranded, aborted, or traditionally harvested animals. Skin fibroblasts were the most common cell type cultured and accounted for 50% (N=13) of tissues studied, followed by kidney epithelial cells 11% (N=3). Mercury (Hg), including methylmercury (CH_3Hg), accounted for 18% of all toxicants (N=6) followed by polycyclic aromatic hydrocarbons (PAHs) 15% (N=5), and organochlorines (OCs) 13% (N=4). In summary, 10% of cetacean species have been investigated, corresponding to 8 genera. We recommend that further research be conducted with emphasis on endangered and declining species in an effort to gain further understanding of how toxicants impact populations.

MP024 Modelling acrylamide acute neurotoxicity in zebrafish larvae

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Acrylamide (ACR) is a water-soluble alkene primarily used in polyacrylamides production. Exposure of humans and laboratory animals to monomeric ACR produces a terminalopathy characterized by ataxia, skeletal muscles weakness and numbness of the extremities. Currently, medical treatment of ACR neurotoxicity in humans is symptomatic, and only the mildly affected patients underwent complete recovery. Similar neurotoxic effects can be found in other species. In order to understand the specific mechanisms of ACR toxicity that could be linked to adverse outcomes across species, we generated a zebrafish model for ACR neurotoxicity by exposing zebrafish 5 days post-fertilization larvae to 1 mM ACR for 3 days. Our results show that the generated zebrafish model mimics most of the pathophysiological processes described in humans and mammalian models acutely exposed to ACR. First of all, the zebrafish model exhibited altered motor function, with a significant reduction in the frequency of the swimming cycles during the escape response. The response to sudden increments or decrements in light intensity elicit was also strongly altered. The histopathological analysis identified an specific effect on the presynaptic nerve terminals at the neuromuscular junctions level, but not on the axonal tracts or myelin sheath integrity. Moreover, the specific effect of ACR on nerve terminals was confirmed by using selected transcriptional markers, as well as by the significant effect found on cholinergic and dopaminergic systems.

MP025 Adverse outcome pathways for selenomethionine exposure in four commercially and culturally relevant Canadian fish species

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Selenium (Se) is a nonmetal atomic element and essential trace nutrient for all domains of life. However, excess consumption of or exposure to various chemical species of Se can have detrimental effects on organisms that may ultimately lead to death of the individual or extirpation of populations. The most environmentally relevant form of Se is the organic form, selenomethionine (SeM). Selenomethionine is generated by primary producers and subsequently bioaccumulates in aquatic food webs, ultimately causing severe teratogenic effects in oviparous animals (ex. fish, birds) as SeM is incorporated into and maternally transferred by the egg yolk protein, vitellogenin. As anthropogenic activities can cause Se influx to the aquatic environment, it is critically important to determine the toxic mechanisms of and species-specific sensitivities to SeM in order to protect vulnerable populations. This research will use microinjected SeM as an analogue for maternally transferred SeM in four commercially and culturally relevant Canadian fish species: fathead minnows (*Pimephales promelas*), white sucker (*Catostomus commersonii*), rainbow trout (*Oncorhynchus mykiss*), and endangered white sturgeon (*Acipenser transmontanus*). Biochemical and apical endpoint analyses will be used to determine the relative developmental sensitivities of these species to SeM and to construct adverse outcome pathways (AOPs) for SeM exposure in each species under study. These AOPs will identify the molecular initiating event (MIE) of SeM toxicity as it pertains to downstream deleterious effects on apical endpoints (survival, length, weight, condition, reproductive output, somatic indices, etc.). Omic (genomic, proteomic, transcriptomic, and metabolomic) analyses, biomolecular assays (glutathione:glutathione disulfide ratio, and thiobarbituric acid reactive substances, superoxide dismutase activity, and catalase activity assays), energetic (triglyceride and glycogen) concentrations of muscle and liver, and histological analyses will be used to help identify the MIE of SeM toxicity in these species. The data acquired and AOPs constructed in this research will subsequently aid in the of regulation of Se in the environment, and aid in the risk assessment of SeM exposure to these fish as well as novel species sharing similar biomolecular characteristics, all while minimizing loss of animal life.

New Approaches to Long-Standing Challenges With Metals: TRV Development and Evaluating Effects in the Field

MP026 Molecular biomarkers from field exposure and their associations with bioaccumulation and sediment bioavailability: Implications for risk assessment

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Due to the complexity of heterogeneous sediment, the chemical speciation and mobility of metals were in dynamic conditions rather than steady states, resulting in high variability of bioavailability. Chemical analyses in traditional tests had disadvantages of inaccuracy for contamination characteristics or risk assessments, representing a worst-case scenario in terms of complex biotic and abiotic exposure dynamics. In this presentation, relevant scientific issues regarding the impacts on metal bioaccumulation and molecular biomarkers with special emphasis on their associations with various metal species were investigated through powerful experimental designs and field-based manipulations of *in-situ* exposure and kinetic DGT approach. Understanding the degree of pollution and geochemical characteristics of sediment matrix, *in-situ* testing chambers were deployed combining the DGT and caged clam *R. philippinarum*, and the dynamic exchanges, translocation and mobilization were then clarified among the interfaces of particles and pore-water in sediments through measuring dynamic parameters and resultant induced fluxes in exposed organism. Integrating a wide battery of biomarkers, dynamic changing processes were assessed, and their interactions were established between exposure and biological effects through multivariate statistical methods. Furthermore, variations of transcript expression of functional genes in contaminant-specific biomarkers were obtained through high-throughput sequencing and quantitative reverse transcription polymerase chain reaction (Q-RT-PCR) in order to elucidate the mechanistic understanding of molecular effects metals induced. Simultaneously, the approach of in-situ evaluation was established considering the consistency of framework as protocols among sediment chemistry, contaminant bioavailability and adverse effect, which significantly improve accuracy and ecological relevancy in complex exposure situation and thus provide a robust tool to support more comprehensive processes of sediment risk assessment. Overall, the associations not only revealed the fates of accumulated metals, but scientifically favored an improved understanding of toxic effects in response to metal bioavailability, supporting the focus on the intracellular processes or events occurring within organism in risk assessment. (The author acknowledge financial support by Grant No. 21377125/B070403 from National Nature Science Foundation of China)

MP027 Using the USEPA Benchmark Dose Software to Develop an Avian Pb Toxicity Reference Value

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Ecological risk and clean-up goals are often based on TRVs developed from NOAELs and LOAELs despite the growing consensus on the inadequacy of these values. For example, the USEPA Region 9 BTAG Avian TRV-L for Pb (0.014 mg/kg-d) is derived from an unbounded LOAEL reported by Edens *et al*. (1976) that was adjusted downward using an uncertainty factor of 10 to estimate a NOAEL. While this TRV may be appropriate for a screening level assessment, the value is overly conservative and not appropriate for development of a clean-up goal. The limitations of TRVs developed from point estimates can be avoided by using dose-response metrics for TRV derivation using programs such as the USEPA Benchmark Dose (BMD) Software. The BMD software fits mathematical models to dose-response data and uses the model output to select a BMD that is associated with a user-defined benchmark response. In this evaluation, the BMD software was used to develop an avian Pb TRV that was not reliant on NOAEL and LOAEL dose levels. The Edens et al. (1976) study was selected because it (1) reported the lowest effect concentration with the most sensitive bird species in the lead Eco-SSL dataset (USEPA 2005), (2) reported all input parameters needed for the BMD model, and (3) was consistent with the study and endpoint (setable eggs) from which USEPA Region 9 BTAG TRV-L was developed. The BMD and BMDL (95% confidence limit on the BMD) were calculated based on a change in the response mean equal to one control standard deviation from the control mean, which is analogous to an EC10, as recommended in BMD Guidance. The high dose data (1000 mg/kg treatment group) was removed to provide better resolution near the low dose levels. Model selection was based on statistical significance, the lowest scaled residual at the control, and the best visual fit around the low doses. The TRVs based on the BMDL and BMD for the best fit model were estimated at 0.15 mg/kg-d and 1.35 mg/kg-d, respectively, which are orders of magnitude higher than the USEPA Region 9 BTAG Avian TRV-L for Pb (0.014 mg/kg-d).

Recent Developments and Current Issues in Bioaccumulation Assessment

MP028 Application of trophic magnification factors (TMFs) under the Water Framework Directive: Some practical advice on selecting and determining a TMF

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Directive 2013/39/EU amending and updating the Water Framework Directive (2000/60/EC) and its Daughter Directive (the so-called EQS Directive: 2008/105/EC) sets Environmental Quality Standards for biota (EQS_{biota}) for a number of bioaccumulative chemicals which can pose a threat to both aquatic wildlife (piscivorous birds and mammals) and human health via the consumption of contaminated prey or the intake of contaminated food originating from the aquatic environment. Member States (MS) of the European Union will need to establish programs to monitor the concentration of 11 priority substances in biota and assess compliance against these new standards for surface water classification. The biota standards essentially refer to fish and should be applied to the trophic level (TL) at which contaminant concentrations peak, so that the predator of the species at that TL is exposed to the highest contaminant levels in its food. For chemicals that are subject to biomagnification, the peak concentrations are theoretically attained at TL 3 to 4 in freshwater food webs and TL 5 in marine food webs, where the risk of secondary poisoning of top predators should also be considered. An EU-wide guidance effectively addresses the implementation of EQS_{biota} (EC 2014). Flexibility is allowed in the choice of target species used for monitoring because of the diversity of both habitats and aquatic community composition across Europe. According to that guidance, the consistency and comparability of monitoring data across MS should be enhanced by adjusting the data on biota contaminant concentrations to a standard trophic level using the appropriate trophic magnification factor (TMF). In this context, the selection of a TMF value for a given substance is a critical issue, since this field-derived measure of trophic magnification can show an appreciable amount of variability, related to the characteristics of ecosystems, the biology of organisms, the physicochemical properties of contaminants, the experimental design, and statistical methods used for TMF calculation, etc. In this presentation, guidance is given for the selection of TMFs for reliable applications within the context of the WFD (i.e. adjustment of monitoring data and EQS derivation). Based on a series of quality attributes for TMFs, a decision-tree is developed to help end users select the “most reasonable” TMF.

MP029 Bioaccumulation of organic contaminants in invertebrates: Challenges ahead

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Invertebrates constitute the biological foundation of numerous terrestrial and aquatic ecosystems. Developing a systematic understanding on how invertebrates bioaccumulate is critical towards establishing a general, mechanistic trophic transfer model for organic contaminants. On-going effort in compiling invertebrate bioaccumulation data has led to the development of a high-quality invertebrate database with detailed documentation on basic bioaccumulation measurements, kinetics, as well as experimental or exposure conditions. The shear amount of data entries present in the database (n > 6000) made new insights possible on the topic through basic quantitative analyses. Further research challenges have also been identified and elaborated. Despite the presence of multiple invertebrate families, both field and laboratory studies have focused heavily on basic bioaccumulation characterization in limited species such as worm, mussel, and clam. Much work remains to be done with respect to other less popular invertebrates.

MP030 Critical Evaluation of an In Vitro Biotransformation Rate Database for Humans

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There is increasing demand to integrate kinetics information into chemical hazard, exposure and risk assessments to better understand relationships between external and internal exposures and to assess bioaccumulation. Despite the fundamental value of biotransformation rate information, relatively few measured in vivo data are available for humans compared to the thousands of chemicals requiring evaluation. Reliable models, in vitro biotransformation rate data, and in vitro-in vivo extrapolation (IVIVE) methods can be applied to address in vivo biotransformation rate data gaps and uncertainty. We have developed a new database of >11,000 human in vitro biotransformation rate estimates (half-lives, clearance rates and rate constants) derived from microsomal, S9 homogenate, and hepatocyte-based assays for >8,500 organic chemicals from the literature and publicly available databases (i.e., ChEMBL). The database is comprised primarily of pharmaceuticals and pharmaceutical candidates from various experimental sources. We developed and applied novel data quality assessment methods based on proposed standardized testing guidance for fish in vitro biotransformation assays to address variability and uncertainty in the database. The data quality assessment methods included compiling physical-chemical property data (e.g., K_{OW}, pKa, water solubility) for all of the chemicals and applying a mass balance in vitro model. The ensuing data quality scores (e.g., high, moderate, or low confidence) may help identify datasets that are most appropriate for QSAR development and for other potential applications (e.g., bioaccumulation screening, prioritization). Calculated in vivo intrinsic clearances ($CL_{IN\ VIVO,INT}$) are compared as case studies. To maximize the utility of the existing and growing body of in vitro biotransformation data, the merits and limitations of the new evaluated database for various use contexts are discussed and key findings of the critical review of existing human in vitro biotransformation rate data are summarized.

MP031 Evaluation of bioaccumulation potential of a super-hydrophobic chemical by dietary exposure bioaccumulation fish test

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Dietary exposure bioaccumulation fish test is prescribed in OECD Test Guideline 305 for poorly water soluble organic chemicals. However, the dietary biomagnification factor (BMF) cannot be determined for a super-hydrophobic chemical when its concentration in fish is too low to measure at the end of the uptake phase or during the depuration phase. In this study, a phenolic compound with estimated $\log K_{ow}$ of 12.7 was subjected to the dietary bioaccumulation test under water flow-through conditions. Fish food containing the test substance of 1000 mg/kg wet weight was fed to fish divided into four groups every day during 28 days. Chemical concentrations in each fish group were analyzed at the end of the uptake phase and ranged from < 0.01 to 0.0135 mg/kg. Since the quantification limit of the chemical was 0.01 mg/kg, it was impossible to determine the elimination rate constant during the depuration phase. Based on the measurements, the lipid-corrected BMF at the 28 days was calculated to be 0.0001. In addition, we tried to estimate the most conservative BMF at the steady state from combination of the measured chemical concentrations in fish and a bioaccumulation mass balance model developed by Arnot et al. With the assumption that the test substance was not metabolized at all in the model, the estimated BMF was sufficiently lower than 1. This result clearly showed that the test compound had a low potential for dietary bioaccumulation in fish.

MP032 Tissue distribution, bioaccumulation characteristics and health risk of antibiotics in cultured fish from a typical aquaculture area

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The negative impacts of residual antibiotics in the environment on ecosystem and human health are big concerns. However, little information is available on the antibiotic bioaccumulation in aquaculture farms. In this study, the bioaccumulative potentials of 28 antibiotics in the plasma, bile, liver and muscle of cultured fish from a typical aquaculture area were systematically investigated. Erythromycin-H2O and trimethoprim were found in all the four tissues. The mean values of log bioaccumulation factors (log BAFs) for the detected antibiotics were in the range of 0.43-4.75, 0.36-4.75, -0.31-4.48, and 0.23-4.33 in the fish plasma, bile, liver and muscle tissues, respectively. For grass carp, ciprofloxacin and enrofloxacin showed high transportability from the plasma to the muscle and liver. The correlations of various antibiotic concentrations between the plasma and the other three tissues were firstly explored. Results indicated that the concentrations of ciprofloxacin and enrofloxacin in the fish tissues could be predicted by their concentrations in the plasma. Human health risk evaluation of antibiotic exposure by fish consumption based on the calculated hazard quotients indicated that the consumption of these cultured fish pose low risks to human health.

MP033 Trophodynamics of Novel Brominated Flame Retardants in the food web of Taihu Lake, South China

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Dispite the increasing usage of novel brominated flame retardants (NBFRs), little is known about their concentrations in organisms and their trophic transfer behaviors in aquatic food web. In this study, concentrations of 2,4,6-tribromophenyl allyl ether (ATE), 1,2-dibromo-4-(1,2-dibromoethyl)cyclohexane (TBECH), tetrabromo-o-chlorotoluene (TBCT), pentabromobenzyl acrylate (PBBA), 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE) were measured in organisms (plankton, invertebrates, and fish) from Taihu Lake, China. Among six NBFRs, strong positive relationships were found between lipid-normalized concentrations of ATE ((trophic magnification factors) TMFs = 8.31, $p < 0.001$) and BTBPE (TMFs = 4.32, $p < 0.001$) and trophic levels, and statistical significance was found for TBCT (TMFs = 7.45, $p < 0.05$) and PBBA (TMFs = 3.16, $p < 0.05$), while increasing trend was found for βTBECH without statistical significance (TMFs = 2.17, $p = 0.07$). For DBDPE, the calculated TMFs value was 0.42 ($p < 0.05$), indicating that DBDPE undergoes trophic dilution in Taihu Lake. The present study optimized in vitro clearance values of six NBFRs to assess the trophic magnification potentials of chemicals in three fish species: yellow head catfish (*Pelteobagrus fulvidraco*), catfish (*Silurus asotus*), crucian (*Carassius auratus*).Chemicals showing significant trophic magnification in Taihu Lake, including ATE and BTBPE, were difficult for biotransformation in all three species. TBCT and PBBA showing trophic magnification, were biotransformed at a different rate among three species($CL/CL_{B[a]P}$: NV-1.59).βTBECH exhibiting no significant trophic magnification, was biotransformed at a moderate rate in three species($CL/CL_{B[a]P}$: 0.1-0.38). DBDPE that undergoes significant trophic dilution in Taihu Lake, was biotransformed at a rapid rate in three species ($CL/CL_{B[a]P}$: 0.43-1.37). The in vitro intrinsic clearance values of the target chemicals among three fish species were found to be consistent with their respective trophic transfer behaviors in Taihu Lake.

In Vitro to in Vivo Extrapolations – Advances and Applications for Risk Assessment

MP034 A combined PBTK and qAOP-modeling approach to assess the impact of DLC-induced embryotoxicity on recruitment failure in European eels

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The panmictic stock of the European eel (*Anguilla anguilla*) has seen a dramatic decline over the past several decades, and declines in recruitment as a result of maternally transferred contaminants has been proposed as one of several potential causes. In particular, dioxin-like chemicals (DLCs) have been identified as a class of chemicals of great concern for both European and American eels (*Anguilla rostrata*). DLCs bioaccumulate, are highly embryotoxic in many species of fish, and maternally transferred in artificially matured eels. However, to date researchers have been unable to locate reproducing adult eels or developing embryos in their natural spawning grounds in the Sargasso Sea. As a result, accurate embryotoxicity data to identify the potential causative chemicals are unavailable. Therefore, this study aimed to (a) parameterize a physiologically-based toxicokinetic (PBTK) model for European eels to account for the impact of changes in physiology that result from sexual maturation and migration on toxicokinetics, and (b) to couple this model with a quantitative adverse outcome pathway (qAOP) for activation of the aryl hydrocarbon receptor 2 (AHR2) of fishes to predict early life stage mortality of eels as a result of exposure to maternally transferred DLCs. The PBTK model was used to kinetically predict the redistribution of DLCs within the body of female eels during migration, and ultimately the concentration in gonads and eggs. A simple qAOP was described previously linking activation of species-specific AHR2 in an in vitro luciferase reporter gene assay using transfected COS-7 cells with embryo lethality across nine species of fishes exposed to DLCs. To this end, AHR2 was cloned from European eel and used to predict eel-specific relative potencies of five DLCs representing congeners measured at among the greatest concentrations in gonads of eels. Using this data, mortality of early life stages of eels was estimated based on the internal concentrations predicted by the PBTK model. Our integrated PBTK model and qAOP approach will ultimately shed light on the question whether early life stage mortality induced by exposure to DLCs has the potential to significantly contribute to the observed decline in recruitment of eels.

MP035 A Quantitative Adverse Outcome Pathway for Activation of the Aryl Hydrocarbon Receptor Leading to Early Life Stage Mortality

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Quantitative adverse outcome pathways (qAOPs) are quantitative, biologically-based models which describe key event relationships that link a molecular initiating event to an adverse outcome. The objective of this research was to develop a qAOP describing the indirect relationship between activation of the aryl hydrocarbon receptor (AHR) and embryo mortality. AHR regulates adverse effects associated with exposure to dioxin-like compounds (DLCs) in vertebrates. Prior investigations demonstrated that sensitivity to activation of the AHR1 (50% effect concentration; EC50) in an in vitro luciferase reporter gene assay was predictive of the sensitivity of embryos (lethal dose to cause 50% lethality; LD50) across all species of birds for all DLCs. However, nothing was known about whether sensitivity to activation of the AHR is predictive of sensitivity to DLCs of embryos of fishes or other vertebrates. This study compared in vitro sensitivities of AHR1s and AHR2s to in vivo sensitivities of embryos of ten species of fish to the model DLC, 2,3,7,8-TCDD. There was a significant linear relationship between in vitro sensitivity of AHR2 and in vivo sensitivity among the investigated fishes ($R2 = 0.97$). The linear relationship between in vitro sensitivity of AHR2 and in vivo sensitivity of fishes was compared to the linear relationship between in vitro sensitivity of AHR1 and in vivo sensitivity of birds. The slope and y-intercept for these linear relationships were not statistically different ($p = 0.11$; $p = 0.052$, respectively). Therefore, all in vitro and in vivo data for fishes and birds across all DLCs was combined into a single significant linear relationship ($R2=0.87$). This simple qAOP was expanded through linking six linear regressions describing the relationship between EC50 for activation of the AHR and no observed effect concentration ($R2 = 0.82$), LD10 ($R2 = 0.90$), LD20 ($R2 = 0.77$), LD50 ($R2 = 0.87$), LD80 ($R2 = 0.71$), and LD100 ($R2 = 0.81$) among fishes and birds across all DLCs. This expanded qAOP can predict full-dose response curves of any DLC for any species of fish or bird, and potentially other oviparous vertebrates. This qAOP will be integrated with a physiologically-based toxicokinetic (PBTK) model in order to predict percent mortality as a result of exposure to maternally transferred DLCs in fishes that cannot be investigated in vivo due to complex life histories.

MP036 Allometric scaling of hepatic biotransformation in rainbow trout

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Biotransformation can markedly reduce the extent to which hydrophobic organic chemicals accumulate in fish. However, predicting the impacts of biotransformation on chemical accumulation is complicated by a number of factors, including the possible influence of differences in fish size. In this study, liver S9 fractions from 4 sizes of rainbow trout (4 – 400 g) were used to evaluate the relationships between fish body weight and the content and activity of phase I and II metabolic enzymes. Phase I activity was assessed by measuring total cytochrome P450 (CYP) content, the activity of CYP1A toward 7-ethoxyresorufin (EROD), and the intrinsic clearance rates ($CL_{IN\ VITRO,INT}$) of 3 polycyclic aromatic hydrocarbons (PAHs). Phase II activity was evaluated by measuring glucuronidation of p-nitrophenol by UDP-glucuronosyltransferase (UGT) and the conjugation of 1-chloro-2,4-dinitrobenzene by glutathione S-transferase (GST). Regression analyses of log-transformed data, expressed on a gram of liver basis, revealed several allometric relationships. CYP content, UGT activity, and GST activity exhibited small but significant inverse relationships with fish body weight. In contrast, $CL_{IN\ VITRO,INT}$ rates for the 3 PAHs increased as body weight increased. However, weight normalized liver mass was also found to decrease inversely with fish body weight. When the data were recalculated on a gram of body weight basis, different patterns emerged. The inverse relationships between fish body weight and CYP content, UGT activity, and GST activity became more pronounced, while $CL_{IN\ VITRO,INT}$ rates for the 3 PAHs showed no significant differences across fish sizes. Overall, metabolic activity varied by less than a factor of 3 across all sizes of fish. To our knowledge, this is the first investigation to systematically evaluate the relationships between fish body weight and metabolic activity of liver enzymes for several substrates and reaction pathways. Results from this study may have important implications for in vitro to in vivo extrapolation of hepatic biotransformation data supporting chemical accumulation assessments for fish.

MP037 Comparing effects between fish embryo and cell models following methylmercury exposure

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There is great interest within the environmental toxicology community in developing alternative testing methods. Benefits of alternative methods such as higher throughput, reduced animal requirements, and lower overall costs than classical toxicity testing methods. Two such alternative methods are the fish embryo test (FET, OECD TG 236), and a zebrafish derived liver cell line (ATCC CRL-2643). To compare these two approaches, well established biochemical assays could be used as endpoints that can be observed in both models. Inclusion of these biochemical assays could potentially deepen their informative scope. The objective of this work is to assess adverse outcomes of methylmercury exposure by combining the toxicity assessments of the FET and cell models with the enzymatic indicators of redox stress. Zebrafish embryos and cells were exposed to a range (2 ppb to 200 ppb) of methylmercury chloride for 96 h with daily solution/medium renewal. The uptake of mercury by embryos and cells was quantified by GC-CVAFS and compared. A suite of apical endpoints was assessed in embryos (embryo coagulation, lack of somite formation, tail non-detachment from yolk, and after 48 h the presence or absence of a heartbeat) according to the FET guidelines. In cells, apical measures included protein concentrations, and viability by resazurin metabolism. In both embryos and cells, enzymatic activity assays included superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase. By the termination of the test, significant occurrences in the FET endpoints were only observed in the highest exposure in lack of tail detachment from yolk, and in the absence of heart beat. In pooled whole embryos homogenates from the FET, there was no significant difference in glutathione reductase activity among treatments. In homogenates from the cell line, there was a significant increase in glutathione reductase activity relative to controls in the medium and high treatments. A comparison of the responses between cell models and embryo models in terms of mercury accumulation, biochemical measures, and apical endpoints will be discussed. This work is expected to deepen our understanding of the advantages and disadvantages of these two alternative model approaches in teleost fish.

MP038 Development of an online HPLC-radiometric detection method for quantification of radiolabeled azole fungicides and pyrethroid insecticides in biota

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The class of pyrethroid insecticides is widely used for control of different insect pests in both agriculture and in private households. They are, however, highly toxic to aquatic invertebrates and have been shown to interact synergistically with different azole fungicides significantly increasing their toxicity. The pyrethroids have traditionally been analyzed by gas chromatography coupled to an electron capture detector or a mass spectrometer (MS) using either electron ionization or negative chemical ionization. Regardless of the sample matrix, pyrethroids are notoriously difficult to analyze due to a number of reasons. They are highly hydrophobic making them sorb to glassware and equipment, they are difficult to ionize for MS-analysis and due to their high toxicity they are only present in the very low ppb range in most samples. To measure exposure and internal biota concentrations of pyrethroid insecticides and azole fungicides in standard aquatic toxicity tests and organisms like *Daphnia magna*, *Chironomus riparius* and *Gammarus pulex* we developed an online HPLC-radiometric detection (LC-RD) method using a Waters Alliance 2695 LC-system coupled to a Hewlett Packard G1314A UV-detector followed by a β-RAM Model 5 detector (LabLogic) with a 500 µL flow cell for radiometric detection. Prior to analysis, the pesticides were extracted from the water phase and up-concentrated by SPE while the QuEChERS technique was used for extraction from the test organisms. The two azoles propiconazole and prochloraz and three pyrethroids lambda-cyhalothrin, cypermethrin and bifenthrin were separated on an Agilent Sorbax Eclipsed XDB-C18 column and the method optimized with regard to chromatographic separation, injection volume, dwell time of the detector and scintillant flow rate with the purpose of minimizing limit of detection which for all five compounds was in the low µg L^{-1} range. Quantification of ^{14}C labelled compounds by LC-RD is not very common but is a promising new technique for especially ecotoxicology and offers several advantages due to the relative ease of use and robustness of the method compared to traditional analysis by GC or LC-MS. In addition, LC-RD is a powerful technique to elucidate new metabolites and we therefore aim to clarify if the test organisms have different biotransformation pathways resulting in formation of different ^{14}C-metabolites in the in vivo studies.

Immunotoxicology – Impacts of Contaminants on Immune Function and Susceptibility to Disease

MP039 Endocrine disruption and immunity: Evidence for altered immune system development following early life stage exposures to endocrine disruptors

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Historically, endocrine disruption studies have focused on a relatively narrow, albeit logical, set of biological endpoints. Studies of estrogenic compounds have generally examined their effects on sexual development and reproduction, while studies of thyroid disrupting compounds have focused on growth and somatic development. However, a growing body of evidence supports a role for hormones in the development and function of the immune system suggesting that it may be a target for disruption by endocrine disrupting compounds. The purpose of this presentation is to: 1) establish a case for the developing immune system as a target for endocrine disruption by providing an overview of the role of endogenous hormones in immune development and 2) to demonstrate the impacts of endocrine disruptors on the developing immune system using data from a suite of recent studies aimed at uncovering the effects of early life stage exposures to the thyroid disrupting compounds on immune function and pathogen resistance. Specifically, the results of experiments demonstrating the ability of propylthiouracil (a thyroid hormone synthesis inhibitor) and polybrominated diphenyl ether 47 (an environmentally relevant thyroid hormone inhibitor) to alter lymphoid development and pathogen resistance will be discussed. Interestingly, the results of these experiments provide evidence that the concentrations required to illicit immune function alterations are lower than those required to reduce somatic growth, suggesting that the developing immune system may be quite a sensitive target for endocrine disrupting compounds.

MP040 Measuring Immune Response in Adult M. beryllina Exposed to Endocrine Disrupting Compounds in Early Life

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Previous studies performed on teleost fish have indicated that endocrine disrupting contaminants (EDCs) have the ability to interact with both estrogen and androgen receptors, which are known to be present in immune cells such as T lymphocytes. Estrogens are demonstrated to generally elevate immune response while androgens act as immunosuppressants. However, there are very few studies that have demonstrated the effects of EDC's on T-cell proliferation in teleost fish when exposed in early developmental stages. Furthermore, there have been no studies performed to determine the potential for cross-generational effects of EDC's on T-cell proliferation in fishes. To address these knowledge gaps we performed a PHA (phytohemagglutinin) immune response assay in order to gauge the effects of EDC's on T cell proliferation in *M. beryllina*. Fish were exposed to levonorgestrel, bifenthrin, ethinylestradiol, trenbolone at low ng/L concentrations, as well as a control (MeOH) (n = 5) until 21 dph.

Once silversides reached reproductive maturity (6-8 months) immune assays were performed on each treatment group with PHA mixed with PBS injections and a control injection of PBS (n = 3-5). Caudal peduncle widths were measured before injection and after 24 hours of isolation post-injection to determine immune response. Preliminary results demonstrated that PBS injections alone, regardless of treatment, were statistically insignificant. In addition, the immune response of the control group was not statistically significant from any of the treatment groups. However, there was a statistically significant difference between bifenthrin and ethinylestradiol ($p = 0.0023$) and bifenthrin and levonorgestrel ($p = 0.0064$). Based on these preliminary results there is more evidence suggesting estrogenic and androgenic EDC's can have differential effects on the immune response of fish. Furthermore, xenoestrogens such as bifenthrin and ethinylestradiol appear to act via different mechanisms, resulting in varied effects on immune response.

MP041 Cyanobacterial toxins affect mammalian innate immunity cells

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Cyanobacteria produce many structurally different biologically active metabolites that are released into the aquatic environment in large quantities. Only a few of them have been studied for their immunomodulatory potency. These toxins may be classified according to the chemical structure as cyclic peptides, alkaloids or lipopolysaccharides (LPS). The aim of the present study is to assess the potential effects of cyanotoxins on macrophages, which represent a crucial part of the innate immune system and initial response to infection. We investigated the effects of the most common microcystin variants (MC-LR, -RR, -YR), cylindrospermopsin (CYN) and the newly isolated aerugenosin (Aer-865) on mouse RAW 264.7 macrophages. We focused on the macrophage activation associated with the production of cytotoxic nitric oxide (NO) and proinflammatory cytokines (TNFα and IL-6). The LPS isolated from gram-negative bacteria was used as a standard macrophage stimulator. Mechanisms beyond the observed effects were explored by studying the uptake of toxins into cells (expression of organic anion-transporting polypeptide transporters, OATPs) and the impacts on activation of intracellular signaling (protein phosphatases, PPs; mitogen-activated protein kinases, MAPKs; and nuclear factor κB (NF-κB)). From the toxins investigated, only MC-LR was able to significantly affect the macrophage responses, while the other MC variants (-RR, -YR) and Aer-865 had no significant effects (non-cytotoxic concentrations tested, i.e. 0.001-1 µM for MCs and 0.1-50 µM for Aer-865). In contrast to MC-LR, CYN alone did not activate macrophages, but we showed that CYN and LPS synergistically activated all studied markers, indicating that LPS exposure sensitizes macrophages to subsequent CYN treatment. Our findings indicate that the immunomodulatory effects of cyanobacterial peptides are highly structure specific and do not depend on the previously recognized mode of action, i.e. intracellular inhibition of PPs regarding MC-LR. Importantly, our results suggested, that the observed effect of MC-LR can be associated with the stimulation of membrane Toll-like receptors (TLRs). Our study contributes to the elucidation of immunomodulatory role of cyanotoxins in macrophages under normal and pro-inflammatory conditions. This study was financially supported by project 16-24949S (GACR); the Czech Republic Ministry of Education, Youth and Sports infrastructure projects No. LO1214 and LM2015051.

MP042 AHR signaling and inflammation profiles in human glioblastoma multiforme cell lines differing in responses to novel experimental AHR ligands

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The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that binds natural, endogenous indole ligands (e.g., indole-3-carbinol from cruciferous vegetables), as well as select environmental xenobiotic toxicants (planar PAHs, PCBs, dioxins). The strength of ligand affinity for the AHR, and the rate of metabolic clearance of the xenobiotic, determines whether its effects lead to toxicity or beneficial outcomes such as balanced immune function and cell cycle control. We determined the effects of two indirubin (indole family) derivatives: Indirubin-3'-(2,3 dihydroxypropyl)-oximether (E804) and 7-Bromoindirubin-3'-oxime (7BIO) on AHR activity and inflammation profiles in LN-18 glioma cells and T98G glioma cells. CYP1B1 gene expression, a marker for AHR activation, is induced in both cell lines, most potently by E804. Inflammation profiles were determined using a commercial cancer-inflammation qPCR array. While CYP1B1 expression is induced by E804, expression profiles of genes indicative of pro-inflammation were highly suppressed by E804, with 7BIO having little effect. This suppression of pro-inflammatory genes by E804 was noted only noted in LN18 cells. Of note, 7BIO reduced the expression of IDO1, a key enzyme important to glioma survival, in LN-18 cells, while this compound increased expression in T98G cells. Moreover, expression of the regulatory transcription factor STAT3 was reduced by E804, but not by 7BIO in both cell lines. Thus, E804 is both an AHR ligand and inhibitor of STAT3. This work is important because gliomas (brain tumors of glial cell origin) are difficult to treat using standard modalities, and they typically suppress immune functions, both systemically and in the local tumor microenvironment. Gliomas also express phase I, II, and III components of xenobiotic metabolism, and thus are useful lines for environmental toxicology, including immunotoxicology. These two glioma cell lines differing in AHR activity levels, though connected with the same cancer type, offer the opportunity to explore the toxicity of a variety of AHR ligands on cell signaling and tumor-related inflammation.

MP043 Synergistic effects of co-exposure to pathogens and pesticides - and the mechanisms behind

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During the last decade we have become aware, that anthropogenic chemicals e.g. pesticides can affect immunocompetences and thereby alter host-pathogen interactions. Pathogens and pesticides may work independent of each other (one not affecting the effect of the other) or they may have interactive (synergizing or antagonizing) effects. The interaction between pathogens and pesticides show great potential for exploitation in integrated pest-management, but unfortunately, the mechanisms of interaction between the two stressors and the host remain poorly understood. Further elucidating the mechanisms is important for the protection of non-target organisms. The aim of the present study (scheduled to commence July-September 2017) is to gain insight into the mechanisms behind effects of pesticides on host immunity and hence on host-pathogen interactions. The model insect host *Tenebrio molitor* is co-infected/exposed with the entomopathogenic fungus *Beauveria bassiana* and the pyrethroid insecticide alpha-cypermethrin. Independence may occur if pesticide metabolism and full damage recovery is reached before or at the time of pathogen exposure, or if the pathways of insecticide detoxification and damage recovery and eliciting an immune response against the pathogen are independent of each other, and if there is no energetic trade-off in eliciting multiple defense responses simultaneously. Synergistic effects may occur from insecticides, which may temporarily reduce grooming behavior, could indirectly increase the establishment success of topically applied pathogens, or insecticide-induced reduction of the insect immune response could lead to faster and/or higher mortality. Pilot studies have shown that a synergistic interaction of pesticide exposure is present during a certain time range of the infection development. To investigate the mechanisms behind the interactions enzymatic activity, internal pesticide concentrations and metabolism (O_2 consumption) will be followed over the time course of disease development in *T. molitor* co-exposed to fungi and pesticide, as well as in individuals exposed to only a single stressor.

Life Cycle Sustainability of Consumer Products

MP044 Approaching to complexity analysis of agrochemical supply for corn-tortilla production in Mexico

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The corn (*Zea mays*) is the most consumed cereal in México mainly as tortilla; even more, tortilla is the most important component in the Mexican diet. Annually, urban communities consume around 56.7 kg per capita, while rural localities consume around 79.5 kg. As a consequence, corn supply for the elaboration of tortilla is always on the verge of shortage. Around the world, corn is obtained by local traditional agriculture, agroindustry, biotechnology or more recently through organic practices. The agroindustry model is the most widespread to produce the corn flour, however industrial crop production damages the environment due to the use of agrochemicals, is cause of erosion, salinization of soils, water pollution, degrades rural communities, impacts farmers' health and compromises animal welfare. This work is an approach since a complexity system to analyze the interconnections in the tortilla production as well as to determine the potential environmental impacts, due to the agricultural production of corn and the corn tortilla making. Our work focuses on the use of pesticides because in Mexico the policy regulation of chemicals follows a model of *soft la*ws which in combinations with other social and environmental factors produces a dire status quo on the key element of the Mexican diet.

MP045 Comparing Regional Household Product Consumption Data for Environmental Exposure Assessments

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Exposure of household cleaning products is based on the use of the product as designed. Consumer product manufacturers conduct risk assessments to ensure safety of their products and to meet regulatory requirements. From an environmental perspective, exposure is often based on assumed down-the-drain emission from consumer use within the household. Options for estimating exposure include utilizing annual tonnage or daily consumer consumption data. Consumption rates may be estimated through consumer habits and practices surveys or market data from third party agencies. This poster will focus on sources of household product consumption data from government authorities and trade associations within North America and the European Union. Opportunities and limitations of the applicability of the source and presentation of the data as well as implications for use in global versus regional environmental exposure assessments will be evaluated.

MP046 Full Life Cycle Assessment study of biomass briquettes for domestic heating purposes: A Lebanese case study

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Energy challenges are global and in the particular case of Lebanon, sustainability issues have been a concern at the national level leading the Government of Lebanon to commit to the UNFCCC in 2009 on increasing its renewable energy shares to 12% of the total fuel mix by 2020. Consequently, the valorization of forestry and agricultural waste through briquetting is currently one of the mostly applied and targeted applications for bioenergy production in the country. As a result, this study focuses particularly on biomass briquettes produced based on olive pruning residues in Lebanon and used locally for domestic heating purposes. It aims at (1) assessing the environmental impacts of a briquette through a full Life Cycle Assessment study, i.e. starting with the collection of raw material until the end of life of the briquette (2) comparing the environmental impacts of briquettes and diesel. In this research, the inventory was modeled using the SimaPro 8.3.0 software, while the IMPACT 2002+ methodology was selected for the Life Cycle Impact Assessment (LCIA) method. The results are very promising; the attributional LCA study showed that electricity consumed during the briquetting production processes is the main contributor to most of the impact categories. From another perspective, compared to briquette, diesel is highly contributing to all impact categories except for "land occupation".

MP047 A life cycle GHG emissions and water intensity assessment of ionic liquid-based biofuel production

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Efficient pretreatment of biomass is important for higher sugar yield and subsequent conversion into biofuels and renewable chemicals. Ionic liquids (ILs) are considered to be a potential alternative to conventional solvents because of their unique properties (e.g., negligible vapor pressure), and their ability to facilitate efficient hydrolysis of lignocellulose to fermentable sugars. However, their greenhouse gas (GHG) emissions-intensity and water footprint are not yet well understood. Therefore, a detailed life-cycle assessment (LCA) is needed to evaluate IL-based pretreatment processes and elucidate opportunities to improve the resulting environmental impacts associated with advanced biofuel production. In this study, we developed a detailed life-cycle model to examine GHG emissions-and water intensity of IL production, specifically Cholinium Lysinate ([Ch][Lys]), and of its subsequent use in a corn stover-to-ethanol biorefinery as a pretreatment solvent. We developed three scenarios: (a) water wash (WW), (b) integrated high gravity - Current (iHG-Current) and (c) integrated high gravity - Projected (iHG-Projected) to explore how IL pretreatment impacts the GHG and water footprint of cellulosic ethanol. Our results show that, depending on the location of lysine production, GHG emissions for [Ch][Lys] production range from 6 to 8 kg CO_2e per kg. For biofuel production, the GHG footprint varies significantly with the biorefinery configuration. For instance, the WW route results in a GHG footprint greater than gasoline, largely because of the energy-intensive IL dehydration step required to recover the IL after the washing step. On the other hand, integrated high gravity configurations have the potential to reduce GHG emissions by ~45% (iHG-current) to ~85% (iHG-projected), relative to gasoline. In terms of water use, IL-based biorefineries are comparable to other, more conventional pretreatment technologies, with water consumption ranging from 0.2 to 0.4 liter/MJ ethanol and withdrawals between 0.4 and 1.2 liter/MJ. The iHG-Projected scenario with >99% IL recovery qualifies for cellulosic biofuel category under the Renewable Fuel Standard (RFS2).

MP048 Sustainability implications of nutrient removal and use in wastewater treatment systems

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Waste water treatment is necessary in an urban setting. Historically, the mission of waste water treatment facilities has been to treat waste to the required standard regardless of the environmental cost. Currently, there is a push to remove nitrogen and phosphorus prior to discharging the effluent, due to their role in the eutrophication of water bodies. This

presents an opportunity to select technologies that will not only remove these nutrients from the effluent, but will also do so in a sustainable manner. This work will focus on struvite recovery (a pelletized form of phosphorus) from the waste water treatment system as a case study in sustainable nutrient management and recovery. Life cycle assessment will be utilized to determine the environmental impact of struvite recovery technology compared to conventional waster technologies with phosphorus discharged into the receiving waters. Multiple impact categories are utilized to present a holistic view of the environmental impact and impacts avoided due to the removal of phosphorus from the effluent stream, and allow for the evaluation of tradeoffs occurring. The resulting struvite product also has the potential to be a consumer product in itself as a slow-release fertilizer. Currently, there is an opportunity to evaluate the environmental impact and sustainability of enhanced nutrient removal processes prior to wide scale adoption in the waste water treatment sector.

MP049 Materials Development at 3M

R. Brown, 3M / 3M Corporate Research Laboratory

3M has 66,000 products which are manufactured, distributed or sold in more than 120 countries; every year, thousands of new products are introduced globally. How 3M meets materials compliance and sustainability requirements in R&D and commercialization impacts our businesses, our supply chain and our customers. As 3M develops new materials, meeting global sustainability and regulatory requirements is a priority. Moving new programs from the research bench to a 3M business involves technical, management, and EHS reviews. The EHS reviews include establishing country specific registrations for chemical ingredients and end product chemistry, assessment of several additional inventories for regulated properties, sustainability evaluations, and recommendations for waste management and export control. Assessments answer 5 questions: Are the materials registered in the countries where they may be manufactured, used or sold? Are there restrictions on handling, manufacturing, use or waste management that must be observed? Are there Import or Export Restrictions? What are the sustainability advantages / concerns? What does the 3M Business need to know to continue commercial development safely and responsibly?

MP050 Evaluation of the Environmental Toxicity and Fate of Sixteen Preservatives used in Cosmetics and Personal Care Products

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Preservatives are added to personal care products (PCPs) to control microbial growth and protect users from pathogens. In the United States, the personal care industry relies on risk-based assessments by the Cosmetic Ingredient Review (CIR) Expert Panel to determine whether preservatives are "safe as used". Due to the potential for repeated and prolonged contact with skin, these safety assessments have been largely focused on human health effects. However, there are increasing concerns about impacts of chemicals in PCPs on the environment following release during use or disposal. At the request of the Environmental Defense Fund (EDF), ToxServices conducted GreenScreen® for Safer Chemicals hazard assessments on 16 preservatives used in PCPs. GreenScreen is a comparative hazard assessment tool that evaluates chemicals across 18 human and environmental health endpoints. The methodology is publically available and involves assigning hazard scores to each endpoint following a review of authoritative lists and toxicity data, and an overall Benchmark™ score that is a high-level indicator of hazard based on combinations of scores for individual endpoints. The environmental toxicity and fate endpoints in a GreenScreen include acute and chronic aquatic toxicity, persistence, and bioaccumulation. ToxServices assessed these endpoints for the 16 preservatives using publically available toxicity data for the target chemicals and surrogates, and modeling when necessary and appropriate. Results of the assessments demonstrate that aquatic toxicity is a hazard for the majority of the preservatives assessed; 12/16 received scores of Moderate or higher for both acute and chronic aquatic toxicity, with 9 and 7 receiving scores of High or Very High for acute and chronic aquatic toxicity, respectively. Most of the preservatives are not expected to be persistent, with only 2/16 receiving scores of Moderate and the remainder receiving scores of Low or Very Low based on evidence that they are rapidly or readily biodegradable. None are expected to present a hazard for bioaccumulation, with all 16 receiving scores of Low or Very Low. While low hazards for persistence and bioaccumulation may mitigate overall environmental risk, questions remain given detection of PCP chemicals in environmental monitoring studies. The identification of preservatives with lower inherent aquatic toxicity hazards is desirable for preservatives with widespread use or release into the environment.

MP051 Development of Derived No Effects Levels from Public Data Sources for Screening Level Human Health Risk Assessment of Cleaning Product Ingredients

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The American Cleaning Institute (ACI) is the industry trade association that represents the formulators of more than 90% of the consumer cleaning products in the United States. Recently, ACI set out to define and publish the universe of ingredients used in its members' products with the goal of identifying the publicly available hazard data for every ingredient and developing an associated exposure assessment and screening level risk assessment. Using a tiered process, a human health hazard data set was sought for almost 600 cleaning product ingredients and 200 supporting ingredients in relevant chemical groupings. Over 7,000 pieces of hazard data were compiled from previous EPA's HPVIS, OECD's HPV chemical archive, ECHA REACH dossiers, FIFRA Inerts Database, TOXNET, EPA's IRIS, previous ACI risk assessments, HERA project assessments, CIR assessments, JECFA, and FDA SCOGS, and other sources. Where data were not available for an ingredient, data gaps were addressed using read-across. Data were filtered by endpoint, test method, reported effect, and test method to select data appropriate for human health assessment. A hierarchical approachwas developed for deriving conservative Derived No Effects Levels (DNELs) for use in risk assessment. Priority was given to ingredient-specific data over read-across data; to data from authoritative multi-source studies versus single studies; to chronic no or low effect levels for sensitive endpoints versus LD50 data from acute studies; and to data with a Klimisch reliability rating of 1 or 2. Where data from defensible sources of chronic NOAELs were unavailable, assessment factors were applied to address uncertainties via conservatism. Where quantitative values were unavailable, qualitative assessments of hazard such as FDA safety findings for high exposure potential food and cosmetics ingredients (e.g., GRAS affirmation) were utilized. DNELs were developed for over 75% of ingredients. This presentation updates previous findings based on the completed hazard data clearinghouse published by ACI; it analyzes trends in data availability per exposure route, data source, and endpoint; reports the methods used for assigning read across; and provides lessons learned.

MP052 Ecotoxicological assessment as a supporting tool for the selection of cosmetic ingredients by industry - Case studies with silicones and surfactants

C. Zacarias, Natura Cosmetics / Product Safety; K. Assanome, Natura Inovação e Tecnologia de Produtos / Product Safety; V. Rocha, Natura Inovação e Tecnologia de Produtos

The reduction and replacement of hazardous substances in products is becoming a global market trend for many industrial sectors. This movement has been caused by a considerable increase in the importance attributed to the chemical sustainability as a potential contributor to reduce the negative impacts industries and businesses may pose to the environment. Agents from academia, governments and from the general public have been increasingly engaged in the assessment of these impacts as well as in the constant proposal of risk management measures. Ecotoxicity is an important indicator for this purpose and its use as criteria for ingredients selection is proposed in the present study. The ecotoxicity impact analysis model, USEtox®, was applied to assess environmentally controversial cosmetic ingredients against potential substitutes. USEtox® is a model based on scientific consensus providing characterization factors for freshwater ecotoxicological impacts of chemical emissions in life cycle assessment, developed under the auspieces of the United Nations Environment Program (UNEP) and the Society for Environmental Toxicology and Chemistry, (SETAC) Life Cycle Initiative. The classes of chemicals included in the present case study were silicones and surfactants. For silicones, a comparison was made between Cyclic Volatile Methyl Siloxanes (CVMS) and Polydimethylsiloxanes (PDMS). For surfactants, the comparison was between sulfate based molecules and other alternatives. To obtain a referential parameter and to determine a degree of criticity in terms of ecotoxicity for each ingredient, the characterization factor was also calculated for Triclosan (known high aquatic toxicity) and Glycerin (known low aquatic toxicity). Our results indicate that the ingredient Sodium Lauryl Ether Sulfate presents the highest ecotoxicity characterization factor, among surfactants. All the surfactants when plotted together with the reference substances Triclosan and Glycerin were positioned in an intermediate range, indicating a moderate ecotoxicity potential. Regarding the silicones, the only CVMS for which a complete assessment was reliably performed was the Octamethylcyclotetrasiloxane (D4), which presented a factor higher than Triclosan. For the group of PDMS, despite the lack of data for USEtox®, the substances present specific physical and chemical properties which do not favor the exposure of aquatic organisms, but rather a prompt elimination in sewage treatment plants.

MP053 Going green- Energy conservation

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Going green does not only involve recycling, it involves much more. It means contributing towards maintaining the natural ecological balance in the environment and preserving the planet and its natural systems and resources. It means to pursue knowledge and practices that can lead to a more environmentally friendly ecologically responsible decisions and lifestyles which can help protect the environment and sustain its natural resources for current and future generations. Going green means to live life as an individual as well as community in a way that is friendly to the natural environment and is sustainable for the earth. Going green means adopting the basic principles: Reduce pollution, Conserve resources, Conserve energy, Reduce consumption and waste and Protect the earth's ecological balance. To put going green in practice, there are many energy saving tips for the offices, homes, schools and wherever we go to. Such practices include: Reduce , Reuse and Recycle. Reduce the use of packaged food items, reuse paper, recycle bottles, plastic containers. Use energy saving bulbs, plant a tree; for every tree that is cut down another one should be replanted. Taking a walk or public transport instead of driving will reduce the toxic fumes being emitted into the atmosphere and also reduce global warming. If we must drive we should use eco friendly cars. All electrical appliances should be unplugged when not in use, go paperless reduce the volume of paper being used as mails in the offices. Use organic homemade products for washing because many of the shampoos, soaps and detergents we use contain chemicals that are washed into the river, ground water and eventually the sea. Grow organic products, make use of compost and avoid fertilizers as they contain heavy metals and genetically modified products. Get involved in activities that will help keep the environment clean and green. The less energy we use, the less pollution we create. When we save energy, our efforts will contribute now and in the long run to keeping our earth and environment sustainable.

MP054 Regulations, Business Models, and Research on Bioenergy from Food Waste in California

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Food waste makes up approximately 15% of municipal solid waste generated in the United States, and 95% is ultimately landfilled. Policy measures are necessary to ensure source-reduction through changes in consumer behavior and improved harvesting, processing, and transportation methods. However, source-reduction alone will not be a sufficient strategy. Food waste that cannot be avoided presents an important opportunity for energy recovery. Research evaluating the food, energy, and water nexus at local and regional levels can help stakeholders identify breakthroughs in technology and policy that provide food security, while enabling economic and sustainable flows of nutrients and energy. These breakthroughs will only be possible if conflicts between recycling, air quality, and energy regulations are resolved, and if business models are developed that lower the risk of bioenergy pathways for food waste producers, waste haulers, as well as organics treatment facility-, wastewater treatment facility-, and power plant manager. This study presents the first detailed analysis of monthly food waste generation in California at the county level, and its potential contribution to the state's energy production. California serves as a useful starting point for building an analysis framework that can be applied to the US or globally because of its diversity and significance in national food production (40% of US vegetables, 20% of dairy, and 70% of fruits, tree nuts, and berry production by revenue). We develop and analyze a set of scenarios that rely on excess capacity at existing anaerobic digester (AD) and solid biomass combustion facilities, and alternatives that allow for new facility construction. Our findings indicate that at least 66% of gross high moisture solids and 23% of gross low moisture solids can be treated using existing local infrastructure, and this fraction increases to 99% of high moisture solids and 55% of low moisture solids if waste can be shipped anywhere within the state. Challenges and opportunities for fostering synergy between business models, regulations, and research on food waste bioenergy will be presented.

Flame Retardants – Sources, Environmental Behavior, Wildlife and Human Exposure, and Effects Implications

MP055 Characterization of Flame Retardant Chemicals in Soils from Urban Gardens

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The testing of soils for contamination has been a topic of research for decades. Most soil contamination studies have historically focused on legacy soil pollutants such as heavy metals, organochlorine pesticides, and petrochemicals and have placed less emphasis on newer organic pollutants. Persistent organic pollutants such as polybrominated diphenyl ether (PBDE) flame retardants have been measured in soil samples around the world, with especially high concentrations being reported in industrial and urban areas, but comparatively little is known about the presence of PBDEs in urban soils in the United States. Within the last decade there has been an increased utilization of urban soils for food production, creating healthier alternatives and offering food security to residents in urban areas. With this increased use of urban areas to garden fresh produce, the potential for human exposure to PBDEs from contaminated soil represents an important knowledge gap, especially in light of the persistence, toxicity, and bioaccumulation potential of PBDEs, together with their potential for translocation from soil into certain food crops. This study seeks to evaluate the presence of PBDEs in soils of urban gardens to better understand the efficiency of garden amendments on the reduction of contamination and the possible risk associated with planting produce in soils contaminated with PBDEs. Soil sampling was conducted at three different urban gardens, two of which utilized native soil, one using bagged soil, in Indianapolis, IN in May of 2017. At each farm, composite samples (n=3-5) were taken both inside and outside of plant beds in order to evaluate contamination at the site as well as the impacts of amendments made within the beds. Purified soil extracts were analyzed for a suite of 36 PBDE congeners (BDEs 7, 10, 15, 17, 28, 30, 47, 49, 66, 71, 85, 99, 100, 119, 126, 138, 139, 140, 153, 154, 156+169, 180, 183, 184, 191, 196, 197, 201, 203, 204, 205, 206, 207, 208, and 209). Concentrations of PBDEs in the urban farm soils will be presented, and the effects of using non-native soil as a mitigation strategy will be discussed.

MP056 Occurrence, Removal and Environmental Emission of organophosphate flame retardants in a WWTP

U. Kim, J. Oh, K. Kannan, New York State Department of Health / Wadsworth Center Department of Health

The occurrence and fate of 14 triester organophosphate flame retardants (OPFRs) and plasticizers and their two diester metabolites were investigated in a wastewater treatment plant (WWTP) in the Albany area of New York State. All target OPFRs were found in wastewater, with average concentrations that ranged from 20.1 ng/L for tris(methylphenyl) phosphate (TMPP) to 30100 ng/L for tris(2-butoxyethyl)phosphate (TBOEP) in influents and from 7.68 ng/L for TMPP to 12600 ng/L for TBOEP in final effluents. TBOEP was the dominant compound in influents (max: 69500 ng/L) followed in decreasing order by tris(1-chloro-2-propyl) phosphate (TCIPP; max: 14500 ng/L), bis(1,3-dichloro- 2-propyl)phosphate (BDCIPP; max: 4550 ng/L), tris(1,3-dichloro- 2-propyl)phosphate (TDCIPP; max: 3150 ng/L) and tris(2-chloroethyl)phosphate (TCEP; max: 8450 ng/L). The fraction of TMPP sorbed to suspended particulate matter (SPM) was 56.4% of the total mass in wastewater, which was the highest among the target chemicals analyzed. The average concentrations of OPFRs in sludge were between 4.14 ng/g dw for tripropyl phosphate (TPP) and 7290 ng/g dw for TBOEP; for ash, they were between 2.17 ng/g dw for TMPP and 427 ng/g dw for triphenyl phosphate (TPhP). The mass loadings of OPFRs into the WWTP ranged from 0.02 mg/day/person for TPP to 28.7 mg/day/person for TBOEP, whereas the emission from the WWTP ranged from 0.01 mg/day/person for 2-ethylhexyl diphenyl phosphate (EHDPP) to 5.12 mg/day/person for TCIPP. The removal efficiencies for OPFRs were slightly above 60% for TMPP, TBOEP and tris(2-ethylhexyl) phosphate (TEHP) whereas those for other OPFRs were < 40% (TPhP and BDCIPP) to negative values, suggesting incomplete removal in WWTPs.

MP057 Occurrence of organophosphate flame retardants in surface and drinking waters from New York State

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The occurrence of organophosphate flame retardants (OPFRs) in surface and drinking water from New York state was studied. The target organophosphate esters were 14 triester and their two diester metabolites: triphenyl phosphate tributyl phosphate, tris(methylphenyl) phosphate, triethyl phosphate, tripropyl phosphate, tris(2-butoxyethyl) phosphate, tris(2-chloroethyl)phosphate, tris(1-chloro-2-propyl)phosphate, tris(2,3-dibromopropyl)phosphate, tris(1,3-dichloro-2-propyl)phosphate, tris(2-ethylhexyl)phosphate, p,p′-1,3-phenylene-p,p,p′,p′-tetraphenylester phosphate, Tri-isobutyl phosphate, 2-ethylhexyl diphenyl phosphate, diphenyl phosphate, and bis(1,3-dichloro-2-propyl)phosphate. Grab samples were collected in pre-cleaned amber glass bottles from June to November, 2016 and from March to June, 2017 covering New York state rivers, ponds, reservoirs and lakes. Totally, 75 surface water and more than 50 drinking water samples were collected. Rain water samples (n=10) were also collected occasionally. To minimize background contamination and systematic error during the sampling and analytical procedure, several quality control samples such as field blanks, travel blanks, laboratory/procedural blanks, and instrumental blank were analyzed. OPFRs were found in a widely in most of the samples analyzed and their profiles varied depending on the samples. On the basis of the concentrations of OPFRs measured in tap water, human exposure doses were calculated.

MP058 Recent changes in PBDE bioaccumulation in freshwater and marine aquatic biota in the Seattle area

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Polybrominated diphenyl ethers (PBDEs) were introduced in consumer products as flame retardants in the 1970s. Their release into the environment, great persistence, and high bioaccumulation rate is reminiscent of polychlorinated biphenyls. Across the United States, accumulation of PBDEs in aquatic life was documented through monitoring surveys. In Puget Sound, PBDEs were found in mussels, fish and marine mammals at high levels. For example, orcas in Puget Sound were documented with some of the highest PBDE concentrations measured in the world. Manufacturers voluntarily chose to stop making octa- and penta-BDE in 2004. Washington State legislature passed a ban on the sale of several common products containing PBDEs in 2008. Soon after, EPA and manufacturers came to agreement to stop producing, importing and selling deca-BDE by the end of 2012. With Washington State being one of the first to restrict all PBDEs, trends in its local waters may be predictive for other states. PBDE declines have been observed but urban areas are slower to respond. King County (in Washington State) has been monitoring fish from Lake Washington (2010 and 2014) and Elliott Bay (2015 and 2017) for PBDEs and other persistent bioaccumulatives. This presentation will summarize the County's fish tissue results for PBDEs in the context of other local data to describe recent changes in PBDE bioaccumulation in the Seattle area.

MP059 Organophosphate Ester Contaminant Metabolism in Convergent Top Food Web Consumers in the Great Lakes: Lake Trout Versus Double-Crested cormorant

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Because of the widespread production, distribution, and commercial use of organophosphate esters (OPEs) as flame retardants, lubricants, and plasticizers, several OPEs have been detected and reported in a variety of environmental media including in biota. OPEs are increasingly being used as replacements for older flame retardants such as polybrominated diphenyl ethers (PBDEs). As a consequence, OPEs are increasingly identified as chemicals of emerging concern and require environmental risk assessments, e.g. under Environment and Climate Change Canada's Chemicals Management Plan. OPE metabolism has been reported for only one wild bird species from the Great Lakes, the herring gull (*Larus argentatus*; which is an opportunistic consumer), where non-halogenated alkyl OPEs (such as triphenyl phosphate) were metabolized in vitro more rapidly than halogenated alkyl OPEs. In the present study, we used a similar liver microsomal assay coupled with quantification of depleted OP triesters and formation of OP diester metabolites using ultra-high performance liquid chromatography triple quadrupole mass spectrometry (UPLC-MS/MS). This study compared OP triester metabolism in vitro in two convergent and top aquatic food web consumers in the Great Lakes: lake trout (*Salvenlinus namaycush*) and double-crested cormorant (*Phalacrocorax auritus*), where the latter is an obligate piscivorous consumer. The choice of OPEs in the present study is based on environmentally relevant OPEs which have been screened for or reported in wildlife and fish in the Great Lakes. The results showed that there are differences in the rate of metabolism, diester metabolite formation, and structure-activity relationships of OPEs as well as there being metabolic differences between the present avian and fish model species. These results provide important information of toxicokinetic processes that can affect the exposure and fate of OPEs in aquatic food web species.

MP060 Identification of Biotransformation Products of Flame Retardant Triphenyl phosphate in Daphnia magna

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Among organophosphate flame retardants, triphenyl phosphate (TPHP), which is alternative material of brominated flame retardants, is processed the usage; TPHP and biotransformation products are found in aquatic environment as micropollutants. TPHP caused adverse effects especially in aquatic organisms but biotransformation research is insufficient. Investigating biotransformation products of TPHP is essential for estimating toxicity in aquatic environment. For identifying the biotransformation products of TPHP, *daphnia magna* was target organism which recommended standard aqueous organism of toxicity study. TPHP was exposed to individual *daphnia magna* for 24 hours. After exposure, *daphnia magna* were homogenized and separated medium were extracted with solid phase extraction (SPE). Samples were analyzed using liquid chromatography-electrospray-high resolution tandem mass spectrometry (LC-ESI-HRMS/MS). Four major metabolites were detected in the study; phase I and phase II mechanisms were applied for investigating the metabolites in *daphnia magna*. Diphenyl phosphate and phenyl phosphate were identified for biotransformation products in phase I metabolites. Among phase II metabolites, sulfonyl triphenyl phosphate and hydroxyl sulfonyl triphenyl phosphate were verified; intermediate metabolites were not significantly detected due to brief retention times. Diphenyl phosphate was estimated in the remaining medium. As minor product, quinone, which is toxic metabolite, was represented to biotransformation product of TPHP in organisms. In conclusion, hydrolysis and sulfation were major mechanisms for biotransformation products of TPHP in *daphnia magna*.

MP061 Flame Retardants and Retrospective Temporal Trends in Polar Bears From the Hudson Bay Arctic

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Flame retardants (FRs) have been widely used over several decades and added to various manufactured materials such as plastics, electronic products and textiles. Legacy FRs include penta/octa bromodiphenyl ethers (BDEs), hexabromocyclododecane (HBCDD), hexabromobiphenyls (PBBs)), which in recent times were listed under Annex A of the Stockholm Convention on POPs. There is increasing use of FR replacements but Arctic biotic measurements and temporal trends data are lacking. Hudson Bay, East Greenland and Svalbard are Arctic contaminant hot spots, and including in polar bears (*Ursus maritimus*), which is a top predator in the marine ecosystem. To our knowledge, no reports exist in the last 10 years on the trends legacy or new FRs in polar bears from any Canadian or Alaskan subpopulation. In the present study, the fat tissue of Hudson Bay polar bears (southern (SHB) and western (WHB) subpopulations) was examined for twenty-five BDE congeners, twenty-four non-PBDE FRs and seventeen organophosphate esters (OPEs). For most years from 1991 to 2016, the temporal trends of quantifiable FRs were investigated. The majority of new FRs, including many of the BDE congeners, were below their respective MLODs, e.g. Dechlorane Plus (DDC-CO) isomers, although Dechlorane 602 (Dec602) and Dec603 were measurable in 56 to 100 % of all bear samples. The only OPE that was quantifiable was TEHP. sumPBDE was composed of BDE47, 99, 100 and 153. sumPBDE, BDE47 and BB153 concentrations were significantly greater in the SHB relative to WHB bears. Age effects were found for sumPBDE, BDE47 and BB153. From 1991 to 2016, sumPBDE concentrations increased up until 2010, followed by an apparent decline to 2016 in WHB bears, whereas a downward trend occurred between 2007 and 2016 in SHB bears. After 2014, HBCDD was not detectable in any fat sample. Statistical examination of the a major BDE congener, BDE47, revealed that for WHB (n=162 bears total) and using a linear regression model there was a +9.0%/year increase (significant, $p< 0.001$) in concentration, whereas using piecewise regression there was a +10%/year increase (not significant, $p = 0.16$) from 1991 to 2003, and a slower +4.3%/year increase (significant, $p< 0.0001$) from 2003 to 2016. For SHB (n=124 bears total) and using a linear regression model only there was a +3.6%/year increase (insignificant, $p=0.45$). Polar bear biomonitoring of FRs needs to continue to better understand sources, transport and fate in the Arctic marine ecosystem.

MP062 HBCDD and TBBPA in the Atmosphere of the Great Lakes

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Hexabromocyclododecane (HBCDD) and tetrabromobisphenol A (TBBPA) are high production volume chemicals that have been widely used as flame retardants. Due to its persistence and bioaccumulative and toxicity properties,the production and use of HBCDD was banned by Annex A to the Stockholm Convention as of 26 November 2014 (UNEP 2014). TBBPA is primarily a reactive flame retardant that is still being used without restrictions. Studies are ongoing to verify its toxicity and its ability to degrade to the harmful BPA and to determine its structural similarity to thyroid hormones. The concentrations of HBCDD and TBBPA in the air (vapor and particle phases) at the five United States Integrated Atmospheric Deposition Network sites located in the Great Lakes basin were measured. These samples were collected from January to December 2014. Preliminary results show varying levels of HBCDD diastereomers. α-HBCDD was found to be the dominant diastereomer in particle samples with an approximate percentage contribution of 19-73%, followed by γ-HBCDD with 16-62%, and β-HBCDD at 8-24%. These data are from Chicago, Cleveland, Eagle Harbor, and Sleeping Bear Dunes. Interestingly, high levels of ∑HBCDD were found at the remote sites, such as Eagle Harbor and Sleeping Bear Dunes (both at about 20 pg m^{-3}). These levels are comparable with those measured at the urban sites of Chicago and Cleveland. TBBPA concentrations were below the instrument limit of detection. These results show that HBCDD is ubiquitous in the air of the Great Lakes.

MP063 A contemporary assessment of polybrominated diphenyl ether (PBDE) in the ambient air and soil of Azerbaijan

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PBDEs were measured in air and soil across Azerbaijan to establish contemporary concentrations at 13 urban and rural sites. Polyurethane foam passive air samplers (PUF-PAS) were deployed for a period of a month with surface soil samples collected at the same sites. Unlike organochlorine pesticides previously surveyed by our group, PBDE concentrations in both contemporary air and soil were low in comparison to recent European and Asian studies. For example, mean $\sum_{10}$PBDE concentrations in air and soil were 26.9±15 pg m^{-3} and 168±57 pg g^{-1}, respectively. Surprisingly, the fully brominated BDE-209 was the most abundant congener observed in both air (25.21 ± 15 pg m^{-3}) and soil (174.8 ± 58.5 pg g^{-1}), comprising ~93% and 96% of $\sum_{10}$PBDE in air and soil, respectively. However, the PAS-derived air concentrations for this congener and other highly brominated congeners must be viewed with caution as there is uncertainty over the uptake rates of particle-bound chemicals using these devices. Some of the highest concentrations in air were observed at sites with the highest wind speeds and at several remote locations in the north of the country and this requires further research. Levels of BDE-47 and 99 (the two most abundant congeners in the widely used penta-formulation) were lower than levels reported elsewhere suggesting limited use/import of the penta-BDE formulation in Azerbaijan.

MP064 Passive air sampling of semi-volatile organic compounds (SVOCs) in Canadian homes using polyurethane foam (PUF) and polydimethylsiloxane (PDMS)

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Indoor SVOCs can occur at much higher concentrations than outdoors, which could lead to elevated inhalation exposures. We assessed a newly introduced PDMS-based passive air sampler (PAS) and the commonly used polyurethane foam (PUF) PAS for measuring indoor SVOCs including novel brominated flame retardants (NBFRs), polybrominated diphenyl ethers (PBDEs), organophosphate esters (OPEs), and phthalates esters (PAEs). Air exchange rates were measured to facilitate the understanding of SVOC levels in household air. Paired PDMS and PUF PASs were deployed for three weeks in bedrooms from 51 homes located in Ottawa and Greater Toronto Area, Canada. PAEs had the highest overall concentrations followed by OPEs (~one order of magnitude less than PAEs) and NBFRs and PBDEs (three orders of magnitude less than PAEs). The dominant compounds (detection frequency > 80) in household air were (median; PUF; PDMS; pg/m^3) ATE (17;16), for NBFRs (85; 63), BDE-47 (47; 27) for PBDEs (100; 58); TCPP (10,800; 3,400) for OPEs (23,000; 5,600), and DEP (130,000; 130,000) and DiBP (170,000; 130,000) for PAEs (670,000; 540,000). Air concentrations measured here were within the range of values previously reported for Canadian homes. Air concentrations derived by PUF and PDMS were not statistically different for gas-phase SVOCs, while PUF had a higher detection frequencies of particle-phase compounds such as OPEs. Exposure of participants to NBFRs, PBDEs, OPEs, and PAEs via inhalation were estimated to be 1.4, 1.6, 370, and 9,300 ng/day.

MP065 Transfer of hexabromocyclododecane from flame-retarded curtains to attached dust

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To examine the transfer of hexabromocyclododecane (HBCD) isomers to dust on the surface of flame-retarded curtains, a series of 196-day laboratory experiments were conducted using two types of curtains and attached dusts. Concurrently, the physicochemical properties (vapor pressure, water solubility, and octanol–water partition coefficient) of the HBCD isomers were measured. HBCD isomers continued to migrate from curtains to dust on a timescale of about 20–50 days. The composition of HBCD, dominated by γ-HBCD in the curtains, was dominated by α-HBCD in the post-experiment dusts, probably because of the higher vapor pressure of α-HBCD compared to γ-HBCD. The initial HBCD contents of the two curtains were comparable, but the concentrations and profiles of HBCD isomers in the post-experiment dusts differed markedly, probably because differences between the texture and/or surface finishing of the treated fabrics affected HBCD transfer to the attached dust. We hypothesized that HBCD vapor from the curtain was sorbed onto dust surface and then gradually diffused into the interior of the dust particles. A mechanistic model based on this hypothesis qualitatively reproduced the observed HBCD concentrations in house dust, lending support to the proposed vapor–sorption–diffusion scenario.

MP066 Using Silicone Wristbands to Measure Individual Exposure to Flame Retardants in Indoor Environment

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Flame retardants (FRs) are used in a large variety of household products, such as furniture, foams, plastics, and electronics. Additive FRs may migrate from host products into indoor environment, leading to potential human exposure. The commonly used methods of assessing human exposure to indoor chemicals (e.g. FRs) include the collection and analysis of indoor air, dust, or human samples (e.g. urine or serum). However, these strategies are limited by the measurement of exposure at a single time point instead of during a period of time or the practicability of collecting human samples in many cases. In the present study, we evaluated the feasibility and reliability of using silicone wristbands to assess integrated exposure of individuals to FRs during a certain period. Volunteers (n = 30) wore wristbands for 15 days and the sorption of FRs on wristbands will be determined. Indoor dust was also collected from the dwellings, offices and cars of participants. Urine samples were collected from participants in the beginning and end of the study period. Samples are being subjected to chemical analysis. The results will be used to test the hypothesis that wristbands can effectively reflect the indoor FR contamination levels as well as human exposure levels by correlative analysis between wristband and dust or urine for the concentrations of FRs or their metabolites. The data will also be useful to develop mathematical models for the prediction of human exposure via wristband as a sampling approach that can effectively represent individual exposure.

MP067 Indoor sources of and human exposure to brominated flame retardants (BFRs), organophosphate esters (OPEs) and phthalate esters (PAEs)

C. Yang, University of Toronto / Earth Sciences; S. Harris, Cancer Care Ontario, University of Toronto / Population Health and Prevention / Prevention and Cancer Control; L.M. Jantunen, Environment and Climate Change Canada / Air Quality Processes Research Section Centre for Atmospheric Research Experiments; D. Tsirlin, L. Latifovic, Cancer Care Ontario; B. Fraser, R. De la Campa, H. You, R. Kulka, Health Canada / Exposure Assessment, Water and Air Quality Bureau; M.L. Diamond, University of Toronto / Earth Sciences

[1]Department of Earth Sciences, University of Toronto, Toronto, ON [2]Population Health and Prevention, Prevention and Cancer Control, Cancer Care Ontario [3]Dalla Lana School of Public Health, University of Toronto, Toronto, ON [4]Occupational Cancer Research Center, Cancer Care Ontario [5]Centre for Atmospheric Research Experiments, Environment and Climate Change Canada [6]Exposure Assessment, Water and Air Quality Bureau, Health Canada [7]Canada Mortgage and Housing Corporation Indoor concentrations of, and exposures to brominated flame retardants (BFRs), and organophosphate esters (OPEs) are influenced by poorly understood sources and, in some cases, poorly understood exposure pathways. Furthermore, the physical-chemical properties of compounds within each class vary greatly, influencing their indoor partitioning and residence time, and hence indoor levels and exposure. Here we report on a study of 51 participants residing in the Greater Toronto Area and Ottawa, Ontario, Canada. Our goal was to characterize indoor concentrations, to identify electronic products as indoor emission sources, and to estimate potential exposures of premenopausal women participants to BFRs and OPEs, and to investigate potential influencing factors related to exposures. Floor dust and indoor air samples were collected from each household environment. Wipes of principal household electronics and participants' hands were also analysed. The median concentrations were used to estimate external exposure of participants to BFRs and OPEs via inhalation, dust ingestion and hand-to-mouth contact. Chemical profiles on a participant's hands resembled profiles found in that person's electronic products. Exposure to these compounds via hand-to-mouth contact equaled or exceeded exposure by dust ingestion. Inhalation intake contributed the least to human exposure of all chemical groups

MP068 Estimating inhalation exposure to flame retardants among Canadian e-waste dismantlers

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The mass of e-waste produced globally is growing dramatically. National numbers suggest that the amount of e-waste dismantled across Canada increased seven times in the period of 2002-2012 from 10,250 to 71,300 tonnes/y. One hazard when handling e-waste are the flame retardants (FRs) which are added to electronic and electrical equipment in order to meet flammability standards. Little is known about FRs exposure to workers in e-waste dismantling facilities in high-income jurisdictions such as Canada. Here, we have undertaken the first study to report on concentrations and profiles of selected FRs in indoor air at an e-waste dismantling facility and estimate inhalation exposure to these FRs among e-waste dismantlers working at a facility in Southern Ontario, Canada. Sampling was conducted daily over a total of five days in February 2017. Polydimethylsiloxane passive air samplers (PDMS-PASs) were hung at 4 workbenches and a central workplace, co-deployed with an active low-volume air sampler which consisted of a GFF followed by a PUF/XAD/PUF sandwich. Each PDMS and low-volume sampler was harvested after 24 hours. Air concentrations of target compounds were used to estimate daily inhalation intakes of these compounds by dismantlers working in the facility. A Micro-Orifice Uniform Deposition impactor (MOUDI) was deployed twice to obtain size segregated air samples. Post-deployment, samples were extracted and analysed for 12 FRs, including novel brominated flame retardants (NFRs), polybrominated diphenyl ethers (PBDEs) and organophosphate esters (OPEs), using gas chromatography mass spectrometry (GC-MS). The most abundant FRs were the now-banned PBDEs (BDE-209 accounted for ~98 % in air collected by PDMS-PASs). The total median concentrations of PBDEs in PDMS-PASs at the central workplace and workbenches were 1930 ng/m^3 and 2900 ng/m^3, respectively. Compared to the previous study conducted in Finland, the levels of total PBDEs in indoor air were about seven times higher. Total daily inhalation intake of 12 FRs among e-waste dismantlers working at workbenches was estimated at ~17 µg/day. Results from the MOUDI showed triphenyl phosphate (TPhP) and other replacement FRs were in PM10, PM2.5 and ultrafine particles in the air of the facility. Levels of FRs in air collected from this Canadian e-waste recycling facility suggest opportunities for inhalation exposure to flame retardants among e-waste dismantlers in Southern Ontario, Canada.

MP069 The investigation of flame retardants in baby food

S. Tang, D. Chen, Jinan University / School of Environment

Flame retardants (FRs) are used in various types of thermoplastics, furniture, electronics, building materials and many other products. After the discontinuation of polybrominated diphenyl ethers (PBDEs) mixtures, additional FRs, including a number of alternative halogenated FRs (AHFRs) and organophosphate FRs (OPFRs), have been subjected to increasing applications. Humans are exposed to FRs via inhalative, oral, and dermal contact pathways. For infants and toddlers, diet may be an important source of contaminants in addition to indoor dust. FR contamination may likely occur during the production, processing and packaging of baby food. Therefore, this study aimed to investigate the levels and compositions of FR contamination in baby food. We selected 40 different brands of baby food (i.e. milk powder, rice flour and dried fruit) manufactured by different countries, including China, the United States, New Zealand, the Netherlands, Denmark, Italy and Singapore. A suite of FRs, including 20 PBDE congeners, 35 AHFRs and 15 OPFRs, were determined in these food items. The chemical analysis is on going and the data will be generated in a few weeks. We expect that our findings will give an insight into the possible FR contamination in baby food and associated daily intake estimate.

MP070 Organophosphorus Flame Retardants in Pregnant Women and their Transfer to Chorionic Villi

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The potential for prenatal exposure has recently raised concerns over the health risks of endocrine disruptors; however, knowledge about human prenatal exposure to organophosphorus flame retardants (OPFRs) is lacking. In this study, 2-ethylhexyl diphenyl phosphate (EHDPP), tributyl phosphate (TBP), triphenyl phosphate (TPHP), and tris(2-chloroethyl) phosphate (TCEP) were detected in the majority of chorionic villus samples, with median concentrations of 13.6, 18.8, 11.1, and 0.51 ng/g dry weight (dw), respectively, significantly higher than those in the matching maternal decidua samples (5.96, 10.8, 1.44, and 0.26 ng/g dw, respectively). The ratios of concentrations in chorionic villi (containing embryos) to those in maternal deciduae (CMRs) were 4.17, 3.82, 2.81, and 2.00 for EHDPP, TPHP, TBP, and TCEP, respectively, which correlated with their logKow values ($p = 0.003$). The results of transthyretin (TTR) binding assays indicated that the stronger the binding ability to TTR, the higher the CMRs were. The median concentrations of the metabolites diphenyl phosphate (DPHP), dibutyl phosphate (DBP), and bis(2-chloroethyl) phosphate (BCEP) were 4.11, 429, and 157 ng/g dw in chorionic villi, higher than those in deciduae (1.64, 181 and 25.4 ng/g dw, respectively). The ratios of DPHP/TPHP and DPHP/EHDPP were 0.20, 0.43 in chorionic villi, and 1.24, 2.03 in deciduae, much lower than those of DBP/TBP and BCEP/TCEP (20.9, 165.6 in chorionic villi and 13.1, 35.3 in deciduae), suggesting that the difference in metabolism between the deciduae and chorionic villi would affect their maternal transfer.

Advances in Environmental Fate and Exposure Modeling

MP071 A Protein-based Model for Bioaccumualtion of Perfluorinated Alkyl Acids in Zebrafish

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Perfluorinated alkyl acids (PFAAs) are man-made industrial chemicals that have highly persistent and bioaccumulative properties in the environment. They are widely used in different consumer and industrial applications. Toxicokinetic studies of PFAAs in both mammals and fish suggest that fate of these chemicals in organisms are influenced by their interaction with various proteins and membrane transporters. Currently no specific mechanistic model exists for the bioaccumulation of PFAAs in zebrafish. The zebrafish, a cyprinoid teleost, is a small freshwater fish and has been considered as an appropriate vertebrate model for investigating developmental toxicity of a wide variety of compounds. Therefore, we have constructed a novel physiologically based pharmacokinetic model that considers the interaction of PFAAs with proteins and transporters in both male and female zebrafish. In addition, our model for the first time considers hepatobiliary circulation. The model comprises 10 compartments: liver, kidney, adipose, muscle, the interstitial fluid between each of these tissues and blood, as well as blood and bile. Most model parameters werecollectedorestimated exclusively from zebrafish-basedstudies. In our model, uptake of PFAAs occurs in the gills via passive diffusion, followed by binding to proteins in blood (probably apolipoprotein in zebrafish, which do not possess serum albumin) and in interstitial fluids of different tissues. Liver contains cytosolic fatty acid binding proteins (FABPs), and PFAAs bind to and dissociate from the FABPs within this tissue. Both passive diffusion and active transport mechanisms contribute to transport of PFAAs in hepatobiliary circulation. We have considered facilitated transport for transport of PFAAs from blood to liver and liver to bile by Apical Sodium-dependent Bile acid Transporter (ASBT), Organic Solute Transporter ?/? (Ost-?/?), and Sodium/Taurocholate

Co-transporting Polypeptide (NTCP) transporter proteins. Through sensitivity and uncertainty analysis, we illustrate the importance of protein interactions and hepatobiliary circulation in the bioaccumualtion of PFAAs in this important model species.

MP072 Predicting Solvent-water Partitioning of Ionogenic Compounds: A Combined pp-LFER/Quantum Chemical Modeling Approach for Environmental Application

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Current fate and transport models for organic chemicals rely largely on linear free energy relationships between environmental systems (i.e., lipid – water, organic carbon – water) and solvent – water systems (i.e., octanol – water) to make predictions of environmental risk and contaminant distribution. Accurate methods for predicting solvent – water partitioning for neutral organic chemicals (e.g., Kow) are well established. However, methods that provide comparable accuracy are not available for predicting the solvent-water partitioning of ionic species. Recent work by Franco et al. has demonstrated for a subset of the 117,000 organic chemicals registered in the European REACH database, approximately 33% have been shown to be "significantly ionized" at environmentally relevant pH values (pH ~ 7.0). Consequently, nearly one-third of the chemical database lacks a predictive model for accurately determining the underlying solvent-water partitioning (and ultimately the fate and transport) of these chemicals in the environment. The purpose of this work is to outline a method of predicting solvent – water partition coefficients for ionic species using Abraham pp-LFER solute descriptors estimated from quantum chemistry. For a suite of carboxylic acid anions, solvent-water partition coefficients for systems: acetonitrile-, acetone-, methanol-, and dimethylsulfoxide-water were predicted with root mean square (RMS) errors of 0.475, 0.512, 0.460, and 0.393, respectively (n = 44, 48, 47, and 41). For a larger set of substituted quaternary amine cations (n = 217), experimentally determined octanol-water partition coefficients were predicted with an RMS error of 1.16. Predictions made using the QCAPs were shown to provide improved accuracy in predicting solvent-water partition coefficients, compared to predictions of solvent-water partition coefficients, made using existing Absolv neutral species solute descriptors. For partitioning of anionic solutes in the four organic solvent-water systems, the overall RMS errors were 0.740 and 0.462 for the Absolv and QCAP methods, respectively. For cations partitioning into octanol overall RMS errors were 0.997 and 1.16, respectively. The QCAP method demonstrates improved accuracy over ab initio quantum chemical calculations at identical levels of theory for both anions and cations (RMSE = 0.462 vs. 2.48 and RMSE =1.16 vs. 2.82) for QCAP-predicted vs. direct QC computed, respectively.

MP073 ppLFER-MUM: Updating the Multimedia Urban Model (MUM) with ppLFERS to model organophosphate esters (OPEs) in Toronto, Canada

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OPEs are a group of chemicals found at relatively high levels as environmental contaminants. The usage of OPEs has increased precipitously in recent years following the listing of penta- and octa- BDEs as POPs under the Stockholm Convention. We modified the Multimedia Urban Model (MUM) of Diamond and co-workers using polyparameter linear free energy relationships (ppLFERs) to estimate the emissions, fate, and transport of three chlorinated (Cl-OPEs) and three non-chlorinated OPEs (non-Cl-OPEs) in Toronto, Canada. Our goal was to estimate their emissions to Toronto and to evaluate their environmental pathways. Aggregate emissions to the air (0-50 m elevation) were estimated by back-calculating from measured outdoor air concentrations. We evaluated these results against measured water concentrations in Toronto tributaries. Based on estimated emissions to air, modelled water concentrations were within an order of magnitude of the measured concentrations, ranging from 88% below for TCPP to 1400% above for TPhP with an RMSE of 400% of the mean measured water concentration. With the exception of TCPP, the model over-estimated water concentrations. Since the water concentrations were taken independently of the air concentrations, these results gives some credence to the model estimates and showed that the emissions estimates were accurate to approximately an order of magnitude. Estimated aggregate emissions to outdoor Toronto air of OPEs for 2010 ranged from 540 (EHDPP) to 7,900 (TPhP) kg/y and were significantly higher than emissions of Σ_5PCBs (240 kg/y) and Σ_5PBDEs (4 kg/y) for 2008, calculated using the same model. We found that generally, Cl-OPEs with their high water solubility, were transferred from air to river water via rain washout and film washoff through storm water, followed by loss from water export (e.g., to Lake Ontario) with minimal accumulation in soils or sediment. In comparison, the less soluble non-Cl-OPEs, with the exception of TBEP, were estimated to be mainly lost via air advection. These model estimates provide evidence of relatively high emission rates to air and, by showing OPE mobility in water, lend credence to the hypothesis of long-range transport of Cl-OPEs by rivers. The major route of transfer for Cl-OPEs to surface water systems is through stormwater runoff, either through film or soil wash-off.

MP074 Applying the RAIDAR model for ecological risk assessment: A case study for 10 organic flame retardants

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Chemicals are being evaluated to determine if they pose unacceptable risks to humans or the environment. Measured (monitored) concentrations in environmental media are non-existent or limited for the vast majority of chemicals produced and used by society. The extensive exposure data gaps hinder the application of risk-based methods for chemical prioritization, screening and comprehensive assessments. The chemical activity approach has been proposed as an integrating concept for chemical risk assessment. The Risk Assessment Identification And Ranking (RAIDAR) model includes multimedia mass balance fate calculations for organic chemicals in air, water, soil, sediment and representative aquatic and terrestrial species and food web bioaccumulation models comprising various trophic levels (e.g., plants, invertebrates, fish, birds and mammals). Here we conduct a case study for screening-level ecological risk assessment using measured and estimated exposure and toxicity data and the RAIDAR model for 10 organic flame retardants (OFRs). The 10 OFRs include chlorinated, brominated and organophosphate flame retardants. The chemicals cover a diverse range of chemical properties for partitioning, reaction, persistence, bioaccumulation and toxicity. A database of 3,120 measured concentrations of 10 OFRs in temperate North America is used to derive emission rate estimates using inverse modelling and to evaluate the model calculations. Estimates of risk are quantified by comparing exposures and effects expressed in terms of chemical activity. Uncertainty in model input parameters (partitioning properties, degradation half-lives, emission rate estimates and toxicity) is used to estimate uncertainty in the RAIDAR risk calculations. A comparative risk assessment is used to rank the OFRs for their relative risks to the environment based on current information for toxicity and chemical emission rates. Comparable risks of OFRs in the environment are partially explained by an inverse relationship between chemical emission rates and overall persistence.

MP075 Predicted Persistence and Response Times of Linear and Cyclic Volatile Methylsiloxanes in Global and Local Environments

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It is important to understand the factors controlling the response times of organic pollutants in environmental systems. In this study, we investigated the response times of eight linear and cyclic volatile methylsiloxanes (VMSs) in environmental systems at different scales from local to global with a particular focus on overall loss rates after cessation of emissions. VMSs have a unique combination of partition properties that are distinct from other organic compounds, including well-established persistent organic pollutants. In particular, they have much higher values of K_{AW} and lower values of K_{OA}. The ultimate environmental sink for VMS compounds overall is the atmosphere, where they react with OH radicals. They are not predicted to be significantly deposited from the atmosphere to terrestrial or aquatic environments so no significant contamination of remote areas is expected. The GloboPOP model estimated very low absolute Arctic Contamination Potential for all the VMS compounds tested and rapid response times were predicted in all media except sediments. VMSs are distributed predominantly in air, where the response time was the shortest due to rapid removal process by degradation. After cessation of emissions the concentrations of VMS in the environment are expected to decrease quickly from current levels. Response times in specific water and sediment systems were evaluated using a dynamic version of the QWASI model and were consistent with those predicted globally by GloboPOP. Response times were sensitive to both chemical-specific properties and characteristics of the specific environmental system considered. Degradation was predicted to play the most important role in determining response times in water and sediment. In the case of hexamethyldisiloxane (L2) and hexamethylcyclotrisiloxane (D3), response times were essentially independent of environmental characteristics because they hydrolyze rapidly in water and sediment. However, response times for other VMSs are system-specific: in environments with high surface area-to-volume ratios (i.e., shallow water bodies) they are relatively short mainly because degradation and volatilization can play an important role in determining response times. In sediment, degradation rates contribute most to the response times. The predicted response times can be used to guide the required sampling frequency to establish changes in environmental concentrations.

MP076 New in silico QSAR models for predicting ready biodegradability of fragrance materials

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One of the key initiatives of the fragrance industry is to boost creation and production of eco-friendly materials. Efforts to synthesize new fragrance ingredients with low environmental impact benefit from use of QSAR models which predict whether a compound is readily biodegradable based on its chemical structure. In this study, we report on the development and validation of a series of predictive models built using a dataset of 472 fragrance ingredients assembled from both public and proprietary sources. The reported models were developed using Optibrium Auto-Modeller and Biovia Pipeline Pilot software packages with different molecular descriptor schemes. These models were benchmarked against the BIOWIN5 and BIOWIN6 models distributed in the EPISUITE software. The new models demonstrated overall prediction accuracy of 0.81 – 0.90 and Cohen's Kappa of 0.56 – 0.71 for randomly selected and structurally diverse hold-out test sets (both ~ 20% of the original dataset). This compares to overall accuracy of 0.74 – 0.79 and Cohen's Kappa of 0.37 – 0.51 for the same test sets when applying the BIOWIN models.

MP077 Soil metabolism of [14C]acetaminophen in two soil types using various soil aliquot sizes

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The OECD 307 Guideline 'Aerobic and Anaerobic Transformation in Soil' and the EPA Guideline OCSPP 835.4100 'Aerobic Soil Metabolism' define the degradation rate of an organic chemical in soil. These guidelines and preceding guidelines like them have been used successfully for years, but questions often still remain concerning the optimum soil size to use in the study and its effect on microbial biomass and the final degradation rate of the test substance. A soil metabolism study design based on the above guidelines was modified in this experiment by setting up treated samples with the pharmaceutical test substance, [^{14}C]acetaminophen, using 3 different soil aliquot sizes with two soil types. Comparisons of the rate of degradation, rate of mineralization and maintenance of microbial biomass have been made between the different soil aliquot sizes among the two different soil types. These results showed that 50 gram and 100 gram soil aliquot sizes provided equally acceptable results in terms of microbial biomass and degradation, and that the smaller 10 gram soil aliquot size did not maintain the microbial biomass as well, even though degradation rates were also similar to the larger aliquot sizes. This study discusses the conditions that lead to a successful test while providing justification of the optimum soil aliquot sizes to be used in future soil metabolism studies.

MP078 Development and application of an agricultural chemicals fate prediction model in Japanese water environment

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In order to ensure the safety of drinking water, many agricultural chemicals are monitored by many of water suppliers in Japan. However, analytical methods for agricultural chemicals in tap water are complicated. Therefore, much labor and cost is required to apply these methods. In the present study, we have developed a 3-D chemical fate prediction model and applied the model to approximately 250 agricultural chemicals, which are the "Complimentary Items" in tap water in the Japanese Waterworks Act. Further, the 3-D chemical fate prediction model was applied to predict detections of agricultural chemicals in the river. This model takes into consideration two forms of agricultural chemicals (particulate-phase and dissolved-phase) in the estuary, and can simulate the diffusion and sinking processes of agricultural chemicals from the loading points of their sources. This model requires flow field data (current velocity, water temperature, etc.), particulate matter (phytoplankton and detritus) concentrations, loading flux, and physical-chemical property of agricultural chemicals (log Koc, and half-life in water) as input data for the calculations. Run-off ratio at the 30 km downstream from loading points and the mass balances of these agricultural chemicals in the river were estimated. The results obtained from this model can be used to select target chemicals for environmental monitoring in Japanese river water. Further, this model can be also applied to human health and ecological risk assessments of agricultural chemicals. The improvement of the model's predictive capability shall be the focus of our next study.

MP079 Comparison of an environmental exposure model to surface water concentrations in Southern Ontario

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The end-of-life of many pharmaceuticals, personal care, and cleaning products is down-the-drain, potentially leading to point-source discharges into rivers and streams. Regulators and the scientific community have an increased interest in understanding the fate and exposure of these chemicals after disposal. Environmental exposure tools can be valuable for assessing the risk of these chemicals to the aquatic ecosystem. Historically, the single medium exposure model, iSTREEM®, only contained geographies within the continental United States. iSTREEM® has been expanded to include the lower St. Lawrence watershed in Southern Ontario. Southern Ontario was selected as a pilot; it is the most populated region in Canada, with a majority of households being connected to municipal wastewater systems. In order to fully understand and most accurately interpret the results of predicted environmental concentrations (PECs) for this new geography, a case study was conducted. The chemicals, triclosan and carbamazepine, were selected as a case study due to their down-the-drain nature of use and the availability of data. Data were extracted representing a 12-year period from Canadian government reports and published literature. The concentrations were compared to the iSTREEM® PECs. Below the 96th-centile, the measured concentrations generally fell between the PECs modeled during low (7Q10) and mean flow. The model provides useful predictions of environmental concentrations of products with a down-the-drain disposal as an end of life fate.

MP080 Towards the development of a framework for applying non-target chemical analysis data within exposure and risk assessment

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There is an increasing trend towards multi-targeted analysis and non-target screening methods to increase the number of analytes monitored in biomonitoring and environmental samples. While the opportunities from advances in chemical analytical capabilities have shown substantive development over recent years, application of information related to data reported from non-target analysis represents a challenge to the field of exposure modelling. For instance, there is no framework for interpreting and using data reported from studies involving non-target analysis to inform exposure and risk. The absence of guidance may consequently lead to difficulties in prioritizing substances for risk assessment or application in exposure estimation. In this study we examine the state of the science with respect to non-target analysis, and present a summary of the merits and limitations in risk prioritization and application in exposure assessment. These preliminary observations are then used to propose an initial framework for the appropriate use of non-target analysis data within exposure assessment. The recommendations concludes with a number of suggestions regarding how these data can be better gathered and reported in order to strengthen their applications for chemical risk assessment, including emerging contaminants.

Fate and Effects of Metals – Biogeochemical Perspective

MP081 Arsenic Speciation in Soils and Sediments: How Data Quality Objectives Determine Most Appropriate Method Selection

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Mobility and bioavailability of arsenic (As) in soil and sediment samples are dictated by the species of As (oxidation state and molecular form) as well as by the matrix-specific constituents that contribute to the physical, chemical, and mineralogical characteristics of the sample. To accurately identify and quantify the As species present in these types of samples, it is critical to select the most appropriate methods for sample preparation and analysis. The various As species have different pH-dependent binding capacities; therefore, the extraction procedure may contribute to matrix-dependent biased data. Furthermore, some detection methods are more susceptible to interferences from elevated concentrations of common soil and sediment constituents, such as iron, manganese, sulfur, and organic compounds, increasing the risk of inaccurate data. A common method historically chosen for arsenic speciation of soils utilizes two different acidic solutions followed by selective hydride generation to facilitate the reaction between arsenite [As(III)] and sodium borohydride to produce a volatile arsine gas. The total inorganic As, monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA) content is analyzed from one of the extracts, while As(III) is analyzed from a different acidic extract and As(V) is calculated as the difference between inorganic As and As(III). Extracts are analyzed via atomic absorption spectroscopy (a modification of EPA Method 1632A). Today, the preferred method for As speciation of soil and sediment samples uses ion chromatography for species separation, followed by analysis using inductively coupled plasma mass spectrometry with collision/reaction cell technology (IC-ICP-CRC-MS). For optimal data quality, two separate extractions, one acidic and one basic, are prepared and both are analyzed for all As species. This fully-validated method allows for the direct quantification of As(III), As(V), MMAs, and DMAs. The speciation results from both extracts are evaluated by an experienced analytical chemist to investigate potential molecular conversion, and the sum of the speciation results is compared to the total As concentration to assess extraction efficiency and mass balance. This presentation provides a brief comparison of these two methods and presents validation data demonstrating how selection of the appropriate analytical method can have a positive or negative impact on achieving the data quality objectives.

MP082 Effects of fragmentation and mercury contamination on marsh periwinkle (Littoraria irrorata) movement

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Habitat fragmentation is currently one of the primary causes of global biodiversity loss. However, not much is known about the interaction effects of fragmentation and contamination. This study performed different laboratory experiments to evaluate how fragmentation and mercury contamination can interact and affect the movement behavior of the marsh periwinkle *Littoraria irrorata*. Random microlandscapes were created using 10x15 inch trays and fragmented with copper tape that repulses the snail. *Spartina alterniflora* blades were set in one side of the tray as to attract the across the microlandscapes. Control and mercury-exposed snail movements were recorded with a digital camera. In the first experiment four different microlandscape patterns were created for three levels of habitat cover (100, 85 and 70 percent of cover). Organisms were exposed to two concentrations of mercury (400µg/L and 450 µg/L) in the water for 96h before the experiment. Twelve organisms were used for each combination of level of cover and mercury concentration. The position of each organism was digitized as x/y coordinates and the mean speed, total distance travelled, and time to start moving were calculated. In the second experiment, one microlandscape pattern was used for six levels of habitat cover (100, 85, 70, 55, 40, and 25 percent of cover). Snails were exposed to two different concentrations of mercury (250µg/L and 350 µg/L). Fifteen organisms were used for each combination of level of cover and mercury concentration. The length and weight of organisms were recorded in both experiments. For each experiment, a generalized liner model with the logit link function was used to evaluate the effects of fragmentation and mercury exposure on the probability of crossing the microlandscapes. In both experiments, fragmentation and mercury had significant effects in the probability of the organism to cross the landscape. The mean speed of organisms was also affected by the mercury exposure but not by fragmentation. These findings suggest that contamination might enhance the effects of fragmentation by affecting the navigation and motion capacity of organisms. Future in situ studies will consider more realistic and long term scenarios.

MP083 Evaluation of utility for stable lead isotopes to identify lead sources and verifying biological fractionation of lead isotopes in goats and chickens

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Stable Pb isotope ratios (Pb-IRs) is widely regarded as an efficient tool for identifying sources and pathways of Pb pollution. However, earlier study revealed large differences in Pb-IRs among biological samples, and that the existence of a threshold for blood Pb levels (Pb-B) for biological fractionation in rats. Hence, the present study was designed to elucidate the presence or absence of Pb-IRs in biological samples, to evaluate the reliability of identifying Pb sources via analysis of Pb-IRs, and to assess whether such threshold was present for goats and chickens in Kabwe mining area, Zambia that has ben well studied, with findings of serious Pb contamination in the environment, animals, and human. Significantly higher Pb level was observed in most of sample types of goat collected from the area close to mining area (G0) compared with those of goat collected from the area 30km away from mine (G30) and 150km away from mine (G150). Similarly, free range chicken (FRC) had significantly higher Pb level in all types of sample than broiler chicken (BC). G150 exhibited a large variation in its Pb-IRs among different tissues supporting the concept of biological fractionation in natural setting. As the Pb levels in the goats increased, the Pb-IRs differences among the tissues and soil became smaller. This finding implies high Pb accumulation might affect biological fractionation. It was revealed the Pb-IRs in all the goat samples are fixed at a certain value when the Pb-B exceeds approximately 5 µg/dL that is considered the threshold for biological fractionation. These findings are quite similar to those from a previous study on rats. In contrary, BC showed small variation in the Pb-IRs although FRC exhibited similar trends to G0 and G30 in terms of their Pb-IRs. These results lead to the assumption that chickens might have negligible biological fractionation, or that the threshold for the disappearance of biological fractionation might be very low in chickens. The Pb-IRs in various tissues from G0 and FRC were quite similar, and close to those of the soil. Although the effect of Pb-B on biological fractionation must be taken into account, Pb-IRs can be still regarded as a reliable tool for identifying Pb pollution sources in the case of mammals having a Pb-B exceeding 5 µg/dL. On the other hand, source identification using Pb-IRs analysis cannot be regarded as being reliable in chickens.

MP084 Fate, speciation, and bioavailability of vanadium in sediments

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Relatively little is known about the bioavailability and speciation of vanadium (V) in sediments despite its occurrence in aquatic environments and increasing production and use. We studied the speciation and bioavailability of V in sediments in laboratory mesocosms and field studies. Surficial sediments were collected from a river reservoir located in the drainage of a former mining area and contained elevated V (range: 240-1620 µg g^{-1}) and other metals. Chemical analyses included V speciation (+4, +5), individual metals, total metal content, Fe/Mn-oxyhydroxide V binding, organic carbon, acid volatile sulfide, pH, and Eh. Chronic (28-day) sediment toxicity tests were conducted using *Hyalella azteca* in field-collected and spiked sediments. Other evaluations included water level fluctuations and reciprocal colonization transplants between reference sites and sites exhibiting elevated sediment metal concentrations. Relative growth rates of *H. azteca* did not significantly correlate to whole sediment or porewater V concentrations or bioaccumulation of any metal. A simulation of reservoir drawdown showed relatively low V concentrations in porewater which did not affect *H. azteca*. While V body burden on *H. azteca* was higher for sites exhibiting elevated sediment V concentrations than reference sediments, there were no associated acute or chronic effects. Porewater Zn exceeded the USEPA threshold for acute toxicity to freshwater organisms. *H. azteca* body burden and growth were negatively correlated for Zn ($p < 0.001$), but not for V. The V LC_{50} derived from sediment spiking was 1738 mg kg^{-1} for *H. azteca* (EC_{10} = 1175 mg kg^{-1}). Despite differences in sediment metal concentrations between sites, metrics for benthic communities on transplanted sediments were similar. Transplanted sediments at sites exhibiting elevated sediment metal concentrations and at reference sites were colonized with pollution-sensitive benthic organisms, suggesting benthic community structure was not impacted by site sediments containing elevated levels of V, Zn and other metals. Our studies show V bioavailability and environmental risk is low despite relatively high sediment metal concentrations, and V is less susceptible to redox changes associated with water level fluctuations than other more labile metals. Although Cr, Ni, and Zn all exceeded sediment probable effect concentrations (PECs), only Zn appeared to have an effect on measured endpoints (*H. azteca* growth).

MP085 Manganese - New Interest in a Common Element

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Manganese is common in well water throughout the world. It is known to contribute to aesthetic issues like black staining of plumbing fixtures, and has a USEPA secondary drinking water standard of 0.05 mg/L based on aesthetics. While treatment of manganese in drinking water is common, it is not currently required for public health protection. However, manganese achieved new notoriety when the Minnesota Department of Health, Massachusetts Department of Environmental Protection, and New Jersey Department of Environmental Protection all nominated it for inclusion in USEPA's fourth Candidate Contaminant List (CCL4) for drinking water. Elevation to this new status may lead to renewed interest in manganese and its properties. The catalytic properties of manganese have been well-known in industrial and water treatment processes for some time. Reexamining what is known about the properties of manganese in light of its possible linkage to subtle neurological effects in children may provide benefit in understanding or managing this risk. Additionally, the growing body of literature regarding the beneficial role of manganese in environmental fate mechanisms for a variety of groundwater contaminants may provide benefit in managing these risks. Manganese therefore represents both an opportunity and a risk for environmental professionals. A variety of existing approaches for balancing the risks and opportunities associated with manganese are available such as greensand filtration, hydrous manganese oxide filters, pyrolusite filters, and permanganate-based oxidation processes. However, there is need and opportunity for new approaches, including the exploitation of manganese oxides in remediation applications and new methods for removal of manganese from drinking water sources.

MP086 Method Comparison for Bioaccessible Lead and Arsenic in Soils

B. Smith, A. Carter, Brooks Applied Labs

Contamination from trace metals, such as lead (Pb), in soils can lead to potential health risks from inhalation and ingestion of the soil or vegetables grown in the soil. Typical risk assessment models assume that 60 - 100% of contaminant metals in a soil sample are bioaccessible. These assumptions can result in incorrect health risk assessments and unnecessarily high remediation costs because actual bioaccessibility can range from 0 - 100%. Currently, in vivo animal studies are often used to determine bioaccessibility, which are expensive and time consuming. *In vivo* methods are giving way to new in vitro methods, such as EPA Method 1340 "*In Vitro* Bioaccessibility Assay for Lead in Soil". The California Department of Toxic Substances Control recently published recommended methodology for evaluating site-specific arsenic (As) bioavailability in soils. This method, the California Arsenic Bioaccessibility (CAB) Method, was developed to improve the accuracy of risk assessments and assist regulators in making responsible remedial decisions while still protecting human health. It is well known that different molecular forms of Pb and As have different toxicological effects; however, the correlation between elemental species in the soil and the bioaccessibility of the metals from that soil has not been well established. For instance, tetraethyllead was historically added to gasoline, while lead chromate, lead oxide, and lead carbonate were common paint pigments. All of these compounds could be present in contaminated soil and each has a different degree of bioaccessibility. For this study, bioaccessible As and Pb concentrations in soil samples were determined using both EPA Method 1340 and the CAB Method, with sample analysis by inductively coupled plasma mass spectrometry utilizing collision/reaction cell technology (ICP-CRC-MS). Both As and Pb were quantified in both extractions to evaluate if a streamlined method could be developed applicable to both elements. In addition, samples were analyzed for total recoverable As and Pb to assess the relative bioaccessibility of each metal in the samples. In order to evaluate if there was a correlation between the bioaccessible metals concentrations and the specific As and Pb compounds extracted by each method, direct quantifications of different molecular forms of the metals (e.g., triethyllead, tetraethyllead, arsenite, arsenate, etc.) were performed in both the soils samples and the bioaccessible metals extracts.

MP087 Spatial variance of dissolved and total Pb within the Cache River Watershed, Arkansas

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Lead (Pb) is an environmental contaminant of great concern due to its many toxic impacts on both humans and other organisms. The Cache River, located in Northeastern Arkansas, has been listed as impaired due to excessive concentrations of dissolved Pb for many years, though the source remains unclear. Here we present a spatial analysis of water samples collected and analyzed over a period of several years (2013-2016) from the Cache River Watershed. Sample collections included smaller, headwater streams and main channel sites in the Cache River and its primary tributary, Bayou DeView. Samples were analyzed with GFAAS for both dissolved (filtered) and total (unfiltered) Pb and final concentrations and frequency of detection were used to create a risk score for sampled sites. Overall trends indicate that for dissolved Pb the greatest concentrations were measured within main channel sites of the Bayou DeView and the Cache River (compared to headwater subwatersheds) with the greatest detection frequency in the Bayou DeView. Overall risk scores indicated that the greatest risk for dissolved Pb was in the Bayou DeView, more specifically in the Caney Creek Subwatershed. For total Pb, a different pattern was observed. Mean concentrations were greater in headwater subwatersheds than in main channel sites. However, the greatest detection frequency was in the Bayou Deview and the most downstream Cache River main channel site. The overall risk score indicated that headwater sites had the greatest risk of contamination with total Pb, although risk was fairly consistent across the watershed. This spatial analysis of risk is useful to identify areas of the watershed most suitable for further assessment and/or implementation of management practices designed to reduce environmental concentrations of both total and dissolved Pb.

MP088 Assessment of pollution levels of some heavy metals in soil and food cultivars from coal mining environment of Enugu State, Nigeria

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Soil and food cultivars in the coal mining communities in Enugu State were investigated for the presence of heavy metals in the soil and food cultivars. Three of the communities are coal mining impacted areas. The heavy metals of soil and food cultivars from these areas were investigated by Atomic Absorption Spectrophotometer. The result of Heavy metal levels in soil of the three communities are within the permissible limit in agricultural soil (Awashthi, 2000), though higher level of Mn, Pb and Cd were observed in Akwuke soil over other communities which could be attributed to anthropogenic and mining activities that took place in the area, and the use of fertilizer. Food cultivars from the three locations were equally proportionately contaminated with these heavy metals. The leafy vegetables (pumpkin and scent leaf) were far more contaminated than the tubers (yam and cassava). This result reveals that coal mining activity is responsible for preponderance of heavy metals in soil and food cultivars of Enugu State. It is suggested that soil and foods remain major endogenous source of heavy metals among the people of the areas.

MP089 Investigation of the relationship between potentially toxic metal (PTMs) concentration in soil and plants along major highways in Lagos, Nigeria

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The possibility of bioaccumulation of potentially toxic metals(PTMs) by edible plants especially those grown along highly trafficked highways poses a threat to humans. This study investigated the levels of PTMs in soil and plants along six major highways in Lagos, Nigeria. The PTM concentrations in the soil and plant were determined by atomic absorption spectrophotometry (AAS) after digestion with aqua regia. Also the physicochemical parameters of the soil were determined by the appropriate standard methods. The results of the analysis showed that lead (Pb) had concentration which ranged from of 14 - 58 mg/kg with a mean of 25.02 mg/kg ± 14.29) for soil the samples and a range of 9 – 42 mg/kg (23.94 mg/kg ± 14.04) for plant samples. Nickel (Ni) and Copper (Cu) had concentrations ranging from 0.03 - 2 (0.61 mg/kg ± 0.43) and 0.3 - 2 mg/kg (0.28 mg/kg ± 0.46) respectively for the soil samples. The levels of Ni and Cu were below detectable limit in the plant samples. The study showed that consumption of edible plants sourcedfrom farms along highway could be a threat to human health due to the possible levels of PTMs that they could contain.

Recent Advances and Future Direction of Per- and Polyfluoroalkyl Substances (PFASs) Research

MP090 Application of the Tissue Residue Approach (TRA) to assess the potential risks of perfluorinated compounds to aquatic organisms

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Perfluoroalkyl acids (PFAAs) are synthetic fluorinated organic compounds that can be released to the environment during manufacturing processes, from commercial products and applications, or indirectly via oxidation of precursor molecules containing perfluoroalkyl chains. While PFAs have received increased attention in monitoring programs, the assessment of the potential risks PFAAs pose to aquatic organisms is still being investigated. The tissue residue approach (TRA) provides a framework for analyzing aquatic toxicity in terms of mode of action and tissue residue concentrations and was applied to perfluorooctane sulfonate (PFOS), perfluorobutane sulfonate (PFBS) and perfluorooctanoate (PFOA) for which toxicity data were available. Using acute and chronic toxicity data from invertebrate and fish studies, and assuming that steady state conditions had been reached at the termination of each study, PFOS TRA values were determined for freshwater organisms. Based on this analysis, calculated whole body TRA values for PFOS ranged from 0.01 to 7.0 mmol/kg. To better understand the uncertainties inherent in this approach, additional analyses were conducted that used kinetic models and/or measured tissue data. This analysis was also applied to PFBS and PFOA. The results of these assessments were compared to PFAA concentrations in fish tissues collected from North America and other global locations. In general, it was determined that current concentrations of these PFAAs do not pose a significant risk to most aquatic organisms.

MP091 Behaviors of perfluoroalkyl acids and their precursors in some sewage treatment plants

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Per- and poly-fluoroalkyl substances (PFASs) have been used for industrial and consumer applications because of their excellent properties such as chemical stability and water/oil repellency, etc. They have also received wide attention due to their persistence in the environment and global distribution. Among them, production and use of perfluorooctanesulfonic acid (PFOS) and its salts perfluorooctane sulfonyl fluoride have been regulated under the Stockholm Convention on POPs. Perfluorooctane carboxylic acid (PFOA) and their related compounds are also phasing out due to the stop their production by their major manufacturers. However, many other perfluoroalkyl acid (PFAA) precursors that may transform into PFAAs in the environment are still being produced. As the precursors are numerous and diverse in their structures, their occurrence in the environment is difficult to grasp by individual analyses. Recently, Houtz and Sedlak (ES&T, Vol. 46, p. 9342-9349, 2012) proposed a new method of detecting total PFAA precursors by their oxidative conversion into perfluoroalkyl carboxylic acids (PFCAs). In this study we used their method to clarify the behaviors and mass balances of PFASs and their precursors in the three sewage treatment plants in Japan. The total concentration of ΣPFAAs (carboxylic, sulfonic and phosphoric acids), increased by 3.8 to 11 times in the influents after the oxidation treatment and indicated that total concentration of PFAA precursors are much higher than that of ΣPFAAs. Among the PFAAs, C_{7-10} aklyl carboxylic acids increased most by the oxidation. While the concentrations of ΣPFCAs did not change much during the secondary biological treatment, the concentration of ΣPFASs including precursors decreased by 70−80%. The removal of ΣPFASs as excess sludge, however, only accounted for 10−20% of the influent loads. This indicated that large removal of ΣPFASs occurred in aeration tanks, possibly by the evaporation of volatile PFAA precursors. The tertiary treatment by chlorination and ozonation did not remove ΣPFASs significantly. Further confirmation, however, is necessary because oxidation of PFAA precursors to PFCAs with corresponding alkyl chain length was indicated to be incomplete.

MP092 Effect of Oxidation Time on the Quantification of Total Perfluoroalkyl Acid Precursors by Potassium Persulfate - Hydroxyl Radical Method

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Per- and poly-fluoroalkyl substances (PFASs) have been receiving attention because of their persistence in the environment and global distribution. Among them, production and use of perfluorooctanesulfonic acid (PFOS) and its salts perfluorooctane sulfonyl fluoride have come to be regulated under the Stockholm Convention on POPs. The production of perfluorooctane carboxylic acid (PFOA), longer-chain homologues and their related compounds is phasing out due to the abandonment of their production by their major traditional manufacturers. Many perfluoroalkyl acid (PFAA) precursors, however, that may transform into PFAAs in the environment are still being produced especially by other developing companies. As the precursors are numerous and diverse in their structures, it is difficult to monitor them individually. Houtz and Sedlak (ES&T, Vol. 46, p. 9342-9349, 2012) has proposed a new method that can determine total concentration of PFAA precursors by their oxidative conversion into perfluoroalkyl carboxylic acids (PFCAs). In this study, we evaluated this method by changing the oxidation time in media with different coexisting organic impurities. N-methyl perfluorooctanesulfonamidoacetic acid (N-MeFOSAA) was selected as an example of precursor and pure water, influent and effluent sewage were used as analyte medium. The oxidation time required to the primary degradation of N-MeFOSAA became longer as the medium had more coexisting organic impurities and still more reaction time than that of complete degradation of the parent compound was necessary for the total concentration of formed PFCAs to reach their maximum. On the other hand, longer oxidation time produced more PFCAs with shorter alkyl chain. Our results indicate that optimum oxidation time to convert all the precursors into corresponding PFCAs differs depending on the coexisting organic impurities in the analytes and perfect conversion of precursor to PFCAs is difficult to achieve.

MP093 Evaluating associations between PFAS in drinking water and human serum in Northern California

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Per- and polyfluoroalkyl substances (PFASs) are a large class of anthropogenic and persistent chemicals, some of which bioaccumulate and are associated with testicular and kidney cancer, high cholesterol, ulcerative colitis, thyroid disease, and preeclampsia. Public concern regarding the ubiquity and potential toxicity of legacy and next-generation PFASs in drinking water has led to increased regulatory pressure requiring more sensitive and selective analytical methods. Our previous study suggests drinking water could be a significant exposure route for PFASs in the general population[1]. To evaluate any association between drinking water and serum PFAS concentrations, here we apply newly developed analytical methods to quantify PFAS in human serum and tap water collected from San Francisco Bay and Sacramento locations in Northern California. Using 0.25 mL of serum and 250 mL of water sample, the analyses were performed by using liquid chromatography (Nexera UFLC system, Shimadzu) coupled to a triple-quadrupole tandem mass spectrometer (SCIEX QTRAP 5500 MS/MS system). In both matrices, we were able to confidently measure 29 PFASs, including 10 out of the 12 analytes listed in EPA method 537: eight perfluoroalkyl carboxylic acids (PFCAs), six telomer acids (TAs), four perfluoroalkyl sulfonates (PFASs), four polyfluorinated phosphate esters (PAPs), three perfluororoctanesulfonamides (FOSAs), two telomer sulfonates (TSs), one perfluoroalkylphosphinate (PFPi), and one perfluoroalkyl phosphonic acid (PFAPA). Our drinking water method has detection limits sufficiently sensitive to comply with the 2016 USEPA Health Advisory Guideline for PFAS in water. Our preliminary data for tap water collected in Berkeley, California, showed average PFAS concentrations ranging between 0.4-1.15 ng/L, with PFOA and PFOS concentrations being highest (mean concentrations of 1.02 and 1.15 ng/L, respectively). Perfluoroalkyl phosphonic acids, polyfluorinated phosphate esters, and long-chain perfluoroalkyl carboxylic acids were also detected. Further investigation on the relationships between drinking water and resident serum for those PFASs detected are underway. Disclaimer: The views expressed herein are those of the authors and do not necessarily reflect those of the Department of Toxic Substances Control [1] Environ. Sci. Technol. Lett., 2016, 3 (7), pp 264–269

MP094 Investigating the metabolic fate of 1H-perfluoroalkanes

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Perfluoroalkyl phosphinic acids (PFPiAs) have been used directly as surfactants for wetting purposes and as anti-foaming additives to pesticides, and have been detected in various environmentally matrices including human blood. In our previous work, PFPiAs were efficiently transformed to 1H-perfluoroalkanes (1H-PFAs) and perfluoroalkyl phosphonic acids (PFPAs) in rats, with over 70% biotransformation after two weeks. Although formed in a 1:1 ratio, the concentrations of 1H-PFAs were higher than PFPAs. The results indicate potential significance of 1H-PFAs as toxic intermediates, however the occurrence and fate of these 6-12 carbon 1H-PFA metabolites is not understood. A study on 1H-perfluoroethane, a CFC replacement, suggested that these longer, 6-12 carbon 1H-PFAs would be biotransformed to a bioactive acyl fluoride intermediate via CYP 450 oxidation. The acyl fluoride may then form either perfluoroalkyl carboxylic acids or may react with biological nucleophiles, resulting in perfluoroalkylated proteins. To test this, 1H-perfluorodecane (1H-PFD) was incubated with rat liver S9 fraction for two hours. Analysis using negative electrospray ionization liquid chromatography-tandem mass spectrometry showed the formation of perfluorodecanoic acid (PFDA), and negative chemical ionization gas chromatography-mass spectrometry was used to determine the decrease in 1H-PFD concentration. An 80% decrease in 1H-PFD was observed in the active experiment compared to the control, decreasing from 7000 ± 1300 nM to 1300 ± 90 nM. After 2 hours, 4.2 ± 0.1 nM PFDA formed, which was 60% above control levels. The formation of PFDA from 1H-PFD only accounted for 0.028% of the 1H-PFD lost over the 2 hours, implying that PFDA was not the only metabolic fate. Currently, work is being done to assess the extent of reactions with proteins. Protein samples from the S9 incubations will be analyzed using total organofluorine combustion ion chromatography to investigate the total amount of covalently bound fluorinated compounds. Chemical proteomics will then be combined with affinity pull down to identify the exact protein targets bound by these bioactive mediates. Understanding the reactivity of 1H-PFAs and their acyl fluoride metabolites will help better understand human exposure to lesser studied PFAS.

MP095 Investigation of sorption of perfluoroalkyl and polyfluoroalkyl substances to soil, pyrogenic carbonaceous materials and soil organic matter

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The environmental fate of perfluoroalkyl and polyfluoroalkyl substances (PFASs) is highly dependent on their mobility. The sorption of zwitteironic, cationic and anionic PFASs relevant to aqueous film forming foams (AFFFs) was studied under varying conditions. While the behavior of anionic surfactants in a soil-water system was in accordance to previously reported studies, the sorption of cationic and zwitterionic PFASs contained in AFFFs was investigated for the first time in five soils of varying physicochemical properties. Non-anionic surfactants displayed increased non-linearity and less consistent K_{oc} values across different soils. In order to further explain the relevant sorption mechanisms of such surfactants, their sorption and desorption were investigated with different solid matrices, such as soil organic matter (Pahokee peat) and three pyrogenic carbonaceous materials (PCMs: biochar, soot, and soot with fuel residues). Batch sorption experiments results showed that sorption to biochar is the strongest of the studied sorbents, while peat is the weakest. Therefore, sorption of PFASs in an AFFF impacted site can be dominated by PCMs rather than soil organic matter. In addition, decreased sorption of PFASs to soot was observed in the presence of oil. This study shows for the first time the mobility behavior of newly identified PFASs in systems relevant to AFFF impacted sites, and demonstrates the importance of the presence of PCMs and oil in such systems.

MP096 Legacy and Replacement Perfluoroalkyl Acids and Precursors and Retrospective Temporal Trends in Polar Bears From the Hudson Bay Arctic

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Per- and poly-fluoroalkyl substances (PFASs) are environmental contaminants, and bioaccumulative perfluoroalkyl carboxylic and sulfonic acids (PFAAs; including perfluorooctane sulfonate (PFOS)) are routinely reported in environmental media. PFOS and PFOA are being replaced by new PFASs such as shorter-chain analogues (e.g. perfluorobutane sulfonate (PFBS)), perfluorooctane-1-ethylcyclohexyl sulfonate (PFEtCHxS) and other precursors of PFAAs. Relative to other circumpolar regions, Hudson Bay, East Greenland and Svalbard are Arctic contaminant hot spots, and including PFASs in polar bears (*Ursus maritimus*), which is a top predator in the marine ecosystem. To our knowledge, there have been no reports in the last 15 years (since 2002) on legacy or new PFASs in polar bears from any Canadian or Alaskan subpopulation, including the climate change hotspot of Hudson Bay. In the present study, the liver tissue of Hudson Bay polar bears (southern (SHB) and western (WHB) subpopulations) was examined for thirteen PFCAs (C_4-C_{18}), four PFSAs (C_4, C_6, C_8 and C_{10}), PFEtCHxS, and PFAA precursors (e.g. perfluorooctane (FOSA) and perfluorobutane (FBSA) sulfonamide). For all years from 2007 to 2016, the temporal trends of quantifiable PFASs were investigated. Over this 10 year period, among all PFASs the concentrations were the greatest for PFOS followed by sumPFCAs (low levels of PFOA, dominated by C_9, C_{10} and C_{11} PFCAs). PFOS and sumPFCA concentrations were influenced by age but not sex, and concentrations were significantly greater for SHB versus WHB bears. Low ppb levels were found for FOSA (PFOS precursor), FBSA (PFBS precursor), PFBA and PFEtCHxS, the first report for any arctic wildlife sample, although no corresponding PFBS was detectable. PFOS and sumPFCA levels showed no obvious increasing or decreasing trends over 2007 to 2016. Statistical examination of the PFOS data revealed that for WHB (n=82 bears total) and using a linear regression model there was a +2.7%/year increase (not significant, p = 0.21) in concentration, whereas for SHB (n=112 total bears) there was a -7.1%/year decrease (not significant, p = 0.16). The lack of temporal change in these bears is despite the PFOS-C_8 chemistry phase-out around 2002 by the 3M Company, and addition to Annex B of the Stockholm Convention in 2009. Polar bear biomonitoring of PFAAs, their precursors and new replacement PFASs needs to continue to better understand sources, transport and fate in the Arctic marine ecosystem.

MP097 Occurrence and Persistence of SAmPAP Triester in Sediments from Taihu Lake: Comparison with SAmPAP Diester

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While (N-ethyl perfluorooctanesulfonamido ethanol)-based phosphates (SAmPAPs) have been proposed as a group of perfluorooctanesulfonate (PFOS) precursors, investigation of their occurrences and fates has been limited to the SAmPAP diester. In this study, concentrations of the SAmPAP triester were determined in samples of sediment from Taihu Lake using a newly developed LC-MS/MS method combined with Envi-Carb cleanup. The triester of SAmPAP was detected in sediments with a detection frequency of 88%, greater than that (56%) of the SAmPAP diester. The mean concentration of the SAmPAP triester was 0.123 ± 0.031 ng/g dry weight (dw), slightly lower than that (0.235 ± 0.111 ng/g dw) of the SAmPAP diester. The SAmPAP diester/triester ratio in sediment was 1.05 ± 4.16, much lower than that observed (6.7) in the technical product purchased in China. In a microbial degradation test of lake sediments, the SAmPAP diester was biodegraded with a half-life of 103 days(95%CI: 63, 248), though the SAmPAP triester was highly recalcitrant to microbial degradation with a half-life of >433 days. Such significant differences in biodegradation would partially explain the relatively high proportions of the SAmPAP triester in sediments. This work, for the first time, reported on the occurrence and persistence of the SAmPAP triester and highlighted microbial degradation of the SAmPAP diester. This work will be helpful to understand potential risk of SAmPAPs and the sources of PFOS.

MP098 Per- and Polyfluoroalkyl Substances (PFASs) Induced Cascade Activation of Plasma Kallikrein-Kinin System in a Structure-Dependent Manner

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Per- and polyfluoroalkyl compounds (PFASs) are a large group of manufactured organofluorine compounds, which have caused global concerns due to their ubiquitous occurrence in human blood. Although the toxicity data on the prevalent forms of PFASs, like perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS), have been extensively reported, the numerous congeners or substitutions with the similar chemical structures represent a poorly understood family of environmental contaminants, whose toxicological properties would be of great importance, considering their increasing commercial production. The potentials of 20 PFASs and the related long-chain aliphatic compounds in activating the plasma kallikrein-kinin system (KKS) have been investigated to evaluate their haematological effects. Three representative PFASs, including PFOA, PFOS and perfluorohexadecanoic acid (PFHxDA) caused dose- and time-dependent plasma prekallikrein (PPK) activation *ex vivo*, which could happen in vivo as evidence by PFHxDA. The studies on the structure-related effects showed that PFASs with longer carbon chain length, higher fluorine atom saturation degree, and terminal acid groups, showed higher potentials in activating the PPK. It was also demonstrated that the waterfall cascade activation of the KKS, initiated by the binding of the chemicals with the zymogen, FXII and its subsequent auto-activation, could be efficiently triggered by PFAS stimulation. The molecular docking analysis showed the binding affinity order of PFASs with FXII was consistent with the capabilities of PFASs in activating the KKS. The findings in this study provided important evidences for the risk assessment on PFAS congeners or substitutions besides the listing in Stockholm Convention.

MP100 Removal of Emerging Polyfluoroalkyl Substances during Drinking-water Treatment

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Per-fluoroalkyl substances (per-PFASs) are anthropogenic chemicals that have been detected in drinking water at numerous places in the United States. The fate of per-PFASs including PFOS and PFOA in drinking-water treatment has been well documented. The USEPA in May 2016 released the updated drinking water advisory on PFOA and PFOS specifying a maximum combined level of 70 ng/L. However, per-PFASs only account for a small fraction of the total organic fluorine in environmental samples. Recently numerous poly-PFASs have been identified. This study focuses on the removal of a group of poly-PFASs during conventional, enhanced, and advanced drinking water treatment, including coagulation, flocculation, sand filtration, activated carbon adsorption, chlorination, and ozonation. The coagulation and flocculation experiments were performed in standard laboratory jar testers with alum (10–120 mg/L) as the coagulant covering both conventional and enhanced coagulation domains. After coagulation, the concentration of poly-PFASs in the supernatant of the settled water was determined. Settled water was then collected and filtered through a sand filter. The sand filtered water was further used in treatment experiments by adsorption via a granular activated carbon (Filtrasorb 200) and a powdered activated carbon (WPL), breakpoint chlorination, or ozonation. It was found that these poly-PFASs are resistant to removal by conventional drinking-water treatment, but can be largely removed from water by activated carbon adsorption. Ongoing experiments are underway to investigate their fate during advanced oxidation processes.

MP101 Seasonal variations and distribution of selected perfluorinated compounds in surface water and sediment from Diep and Plankenburg rivers, Cape Town

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Seasonal variation and distribution of nine perfluorinated compounds (PFCs) in surface water and sediment samples from rivers in Cape Town, using ultra performance liquid chromatography couple with quadrupole time of flight (UPLC-MS/QTOF). Maximum concentrations of PFCs were recorded during the summer period in both surface water and sediment samples. Generally, levels of PFCs in surface water and sediment samples were below published established threshold limits. From our findings, concentrations of PFOA ranging between ?LOD and 153.5 ng/g dw) and PFOS ranging between ?LOD and 246.25 ng/g for the sediments samples, and level of PFOA ranging between OCvalues for PFOA and PFOS in the river system were 1.311 and 1.316 cm^3/g respectively.

MP102 Trends in PFAS in Sport Fish and Birds in San Francisco Bay, California, USA

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Based on the results of monitoring San Francisco Bay biota, sediment, water, effluent and stormwater for over a decade for per- and polyfluoroalkyl substances (PFASs), the Regional Monitoring Program for Water Quality in San Francisco Bay (RMP) has classified perfluorooctane sulfonate (PFOS) as a contaminant of moderate concern. PFOS and perfluorooctanoic acid (PFOA) have been widely detected in humans and in the environment. As such, these chemicals and related compounds have been the subject of regulatory scrunity in the US, Canada, and Europe with the industry now favoring shorter-chained compounds. To evaluate the efficacy of the regulatory actions, the RMP has placed a high priority on monitoring bird eggs and fish for PFOS and related PFASs. Double-crested cormorant (*Phalacrocorax auritus*) eggs have been monitored for 13 PFASs from three locations in the San Francisco Bay on an approximate triennial basis since 2006. Concentrations of PFOS in 2006 and 2009 were some of the highest concentrations observed worldwide (1,000 ng/g); recent results from 2012 and 2016 indicate declines. Interestingly, concentrations of the longer-chained perfluoroalkyl carboxylates such as PFOA, perfluorodecanoate (PFDA), and perfluorododecanoate (PFDoDA) do not show similar declines. The RMP has monitored sport fish at five locations in San Francisco Bay for PFASs twice, in 2009 and 2014. Concentrations of PFOS remain below the Minnesota Department of Health guideline for consumption of contaminanted fish in the main portion of the Bay. Recent sampling in closer proximity to the potential sources indicates elevated concentrations of PFOS relative to the Bay sites, and the first detections of a number of longer-chained perfluoroalkyl carboxylates such as PFDA, perfluoroundecanoate (PFUnDA) and PFDoDA. The presence of these longer-chained carboxylates in fish and bird eggs suggests ongoing sources.

Implementation of TSCA as Amended by the Frank R. Lautenberg Chemical Safety for the 21st Century Act – Science Issues

MP103 Environmental Toxicity Data Collection Process, Criteria and Challenges Under the Amended TSCA

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On June 22, 2016, the Frank R. Lautenberg Chemical Safety for the 21st Century Act was signed into law thereby amending the Toxic Substances Control Act (TSCA). Implementation of the amended legislation has resulted in changes to how ecological risk assessments are conducted by the Office of Pollution Prevention and Toxics (OPPT). For risk evaluation of the first 10 priority chemicals under the existing chemicals program, EPA is developing methodologies to systematically search the literature for environmental hazard data and information. A systematic process for collection of environmental hazard information includes criteria and methodologies for selection and evaluation of data/information. OPPT modified the existing ECOTOX literature search protocol for acquisition and data extraction used by the Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division. The modified process includes a wide search of all literature holdings, including grey literature, conducted using chemical specific search terms, followed by title and abstract screening using applicability criteria adapted for amended TSCA, and finally, application of modified acceptability criteria to further screen the literature.

One challenge has been to harmonize the ECOTOX literature search strategy and results with the process developed for fate, human health, engineering and exposure literature searches. For example, the first screen of ECOTOX literature search results usually tag references as “excluded”, “applicable” and “acceptable” whereas EPA decided to use “on-topic” and “off-topic” for the TSCA priority chemical assessments. Another challenge has been to adapt the ECOTOX search and screening criteria to fulfill TSCA requirements, for example, to include literature with in vitro methodologies. Further details and challenges of developing a systematic data collection and evaluation process to fulfill amended TSCA needs will be discussed. Disclaimer: The views expressed in this abstract are those of the authors and do not represent USEPA policy or endorsement.

MP104 Super-hydrophobic chemicals and potential risk to sediment-dwelling organisms

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The Toxic Substances Control Act (TSCA) as amended by The Frank R. Lautenberg Chemical Safety for the 21st Century Act allows for regulation of chemical substances which may present unreasonable risk of injury to human health or the environment. Chemicals which are considered super-hydrophobic with log Kow values > 7 (e.g., long chain alkanes, chlorinated alkanes, some halogenated aromatics, phthalate esters, and brominated fire retardants) are difficult to test due to factors such as extremely low water solubility, high sorptive capacity (partitioning), and bioaccumulation potential. These factors render the use of standard water column tests problematic for assessing chemical toxicity. As an alternative to water column-based testing, sediment tests offer several advantages by incorporating multiple exposure routes (water, food, sediment), improving the ability to quantify exposure via sediment concentrations and allowing for a wider array of standard invertebrate test species. In this project, we characterize the exposure, effects, and potential risks to sediment-dwelling organisms for some super-hydrophobic substances using data from the open literature and exposure models to characterize the “value added” of sediment testing for addressing the unique challenges presented by these hydrophobic chemicals. Disclaimer: The views expressed in this abstract are those of the authors and do not represent Agency policy or endorsement.

MP105 An Approach to Estimate Ecological Risks to Higher Trophic-Level Organisms to Hexabromocyclododecane (HBCD)

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On June 22, 2016, the Frank R. Lautenberg Chemical Safety for the 21st Century Act was signed into law thereby amending the Toxic Substances Control Act (TSCA). Implementation of the amended legislation is an on-going process which will result in changes to how ecological risk assessments are conducted by the Office of Pollution Prevention and Toxics over time. This poster will provide an overview of an approach to estimate ecological risks to higher trophic level organisms to hexabromocyclododecane (HBCD). HBCD was one of the first ten chemicals identified for risk evaluation under the revised TSCA in 2016. HBCD is a brominated flame retardant largely used in the construction industry in expanded polystyrene foam (EPS) and extruded polystyrene foam (XPS). HBCD is classified as a persistent, bioaccumulative, and toxic (PBT) chemical with biomagnification properties. An ecological risk assessment is conducted to evaluate and determine how likely it is that the environment may be impacted because of exposure to a chemical once it is introduced to the environment. For HBCD, existing ecological toxicity studies suggest there are hazards for many species including *Daphnia magna*, a water flea, and multiple fish and bird species. *Daphnia magna* are the most sensitive species to HBCD. While fish appear less sensitive to HBCD, HBCD has been detected in many ecologically relevant fish and bird species. Since HBCD has the potential to bioaccumulate and biomagnify in both aquatic and terrestrial organisms, we explored a higher trophic-level approach to the ecological risk assessment of HBCD using the predator-prey relationship of osprey (*Pandion haliaetus*) and fish. This analysis revealed that if osprey are eating fish (e.g., European Flounder (*Platichthys flesus*) or Rainbow Trout (*Oncorhynchus mykiss*)) at concentrations observed in monitoring data, osprey may be consuming concentrations that could result in hazardous effects. This concern is further demonstrated by osprey egg monitoring data, which show HBCD concentrations in osprey laying females at and above the adverse effect concentrations. This case-study identified the value of incorporating an approach to estimate ecological risks to higher trophic level organisms for chemicals that bioaccumulate and biomagnify in the food chain. The views expressed in this abstract are those of the authors and do not represent Agency policy or endorsement.

Development and Application of Biologically Based Mathematical Models in Adverse Outcome Pathways (AOP)

MP106 A Framework for Linking Population Model Outcomes with Ecological Risk Assessment Objectives

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The value of models that link organism-level impacts to the responses of a population in ecological risk assessments (ERA) has been demonstrated extensively over the past few decades. There is little debate about the utility of these models to translate multiple organism-level endpoints into a holistic interpretation of effect to the population; however, there continues to be a struggle for actual application of these models as a common practice in ERA despite the well-documented scientific basis. While general frameworks for developing models for ERA have been proposed, there is limited guidance on when models should be used, in what form, and how to interpret model output to inform the risk manager’s decision. We propose a framework for developing and applying population models in regulatory decision making that focuses on tradeoffs of generality, realism, and precision for both ERAs and models. We initiate the framework development with a conversation between regulators and modelers aimed at defining the added value of specific types of model output relative to the assessment objective. We explore why models are not widely used by comparing their requirements and limitations with the needs of regulators. Using a series of case studies under specific US regulatory frameworks (e.g., FIFRA, CERCLA, etc.), we classify ERA objectives by tradeoffs of generality, realism, and precision and demonstrate how the output of population models developed with these same tradeoffs informs the ERA objective. We discuss attributes for both ERA and models that can be used to classify each with respect to these trade-offs. The proposed framework will assist risk assessors and managers in identifying models of appropriate complexity and understanding the utility and limitations of the model’s output and associated uncertainty in the context of their assessment goals.

MP107 Computational Modeling of the Effect of Perfluorooctanic Acid on the Survival of Loggerhead (Caretta Caretta) Sea Turtle Cells

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Little toxicological research has been conducted on endangered loggerhead sea turtles (*Caretta caretta*). In this study, targeted in vitro viability data was collected from loggerhead skin cells exposed to perfluorooctanic acid (PFOA). PFOA is a known marine contaminant with documented detrimental effects in reptiles and existing tissue burdens in loggerheads. Data collected parameterizes a toxicokinetic and toxicodynamic (TKTD) model. TKTD modeling has two modeling approaches for survival: stochastic death (SD) and individual tolerance (IT). In SD models one toxic concentration threshold is identical for all individuals. When the internal concentration in the organism exceeds this threshold, the predicted survival decreases equally among individuals. IT models are taking into account inter-individual sensitivity to toxicants, where the response to the stressor varies among individuals. Including both SD and IT model approaches is a novel modeling technique. Parameters created from our viability data was used to predict the lethal effects of PFOA on loggerhead cells over time. This is the first report of any model applied to infer the survival of sea turtle cells exposed to a marine contaminant. Effectiveness of both SD and IT models will be further analyzed using profile likelihoods, confidence intervals, and r-squared. This study creates a paradigm for toxicological studies in sea turtles by extrapolating the toxic effects from cells to individual sea turtles, using a survival likelihood endpoint, and assessing the use of a less-invasive tool to monitor toxicological effects of marine contaminants in an endangered marine species.

MP108 Mechanistic modeling of chemical stressors: Impacts of estrogens (EE2) on the greenback cutthroat trout

C. Accolla, V. Forbes, University of Minnesota / Ecology, Evolution, and Behavior

Ecological risk assessment ought to make predictions about the likely effects of chemical contaminants on populations and ecosystems. However, current approaches often fall short because methods for estimating and integrating exposure and effects are often based on overly simplistic assumptions. Indeed, management decisions are based on a very limited number of species that can be easily cultured and kept in the lab, whereas the final goal is generally to protect populations, communities and ecosystems. Moreover, we often underestimate the role of trophic relationships in ecosystems exposed to chemicals and other stressors. Mechanistic models have an essential role to ensure better predictive capacity, integrating the effects of chemicals across multiple levels of ecological organization. Furthermore, ecological models can extrapolate both stressor impacts and life histories across species that cannot be directly tested in the lab. The aim of this research is to develop an individual-based model (IBM) of an endangered trout species, the greenback cutthroat trout (*Oncorhynchus clarkia stomias*), to test possible population-level impacts of exposure to 17α-ethinyl estradiol (EE2). The individual description is based on Dynamic Energy Budget theory. DEB theory describes the metabolic organization of organisms and is a standardized theory, tested for many species and in different contexts. This DEB-IBM has also some extra mortality terms for taking into account the weak recovery rate of the greenback cutthroat trout. In particular, we represent competition with the brook trout (*Salvelinus fontinalis*), which seems to affect egg and juvenile survival rates. Using this approach, it will be possible to represent individual variability, such as different reproduction/mortality rates, changes in behavior or different responses to stressors. Simulation results will eventually allow to infer the consequences of EE2 exposure at the population level under various levels of competition and other ecological influences. This is particularly relevant in the context of ecological risk assessment, since it is crucial to understand the linkage between chemical exposure and an adverse outcome at individual and higher levels of organization.

MP109 Physiologically Oriented Interactions of Nutrients and Toxicants (POINT) Model Demonstration of Mercury-Selenium Biochemical Interactions

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Assessing effects of toxicants such as methylmercury (MeHg) whose mode of action depend upon bimolecular reaction mechanisms require concurrent assessments of their kinetics as well as dynamic interactions with their target species. Selenium (Se), is an essential trace nutrient that is required for synthesis of selenocysteine (Sec), the 21st genetically encoded amino acid. Since Sec is required at the active sites of ~25 genetically & functionally unique enzymes expressed in human tissues. Since selenoenzymes perform numerous brain functions, excessive exposures to MeHg, the only environmental insult known to irreversibly inhibit their activities, are harmful, especially during fetal development. Since the toxic effects of high MeHg exposures can be prevented by increasing dietary Se intakes, including those from eating Se-rich ocean fish, MeHg exposure from fish consumption is proportional to risk only in situations where MeHg occurs in molar excess of Se such as in meats of top predators such as great white and mako sharks or predatory whales. In this study, physiologically oriented interactions of nutrients and toxicants POINT) models are used to predict chemical, biochemical, physiological, and neurological effects of different intakes of Se and MeHg as observed in a series of rat feeding trials. POINT models applying the recently updated Health Benefit Value (HBV) seafood safety criterion were found to reliably predict the brain levels of: Hg, Se, thioredoxin reductase (TRx) glutathione peroxidase (GPx) and F2-isoprostane (an indicator of oxidative damage). In animals with low dietary Se intakes, brain TRx and GPx activities were severely impaired by MeHg exposures, but increasing dietary Se from delipidated ocean fish protein increasingly counteracted the inhibitory effects of high MeHg exposures as predicted by the POINT model. Oxidative damage indicated by F2-isoprostane in the brains of MeHg exposed rats was inversely related to brain GPx and TRx, indicating that the oxidative damage known to be associated with high MeHg exposures is due to loss of selenoenzyme activities. This study confirms that dietary HBV is a superior index of risks due to high MeHg exposures. Because the HBV provides a direct reflection of the bimolecular reaction mechanisms associated with MeHg toxicity, it provides a more reliable approach to risk assessment than criteria based on MeHg exposures alone. The POINT model will soon be applied to evaluate human study data.

MP110 Scaling up endocrine disruption effects from individuals to populations: Outcomes depend on how many males a population needs

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Assessing how endocrine disrupting compounds (EDCs) affect population dynamics requires tracking males and females (and sex-reversed individuals) separately. A key component in any sex-specific model is the 'mating function', which is the relationship between sex ratio and reproductive success, but this relationship is often unknown. As such, we quantified this relationship empirically in the inland silverside, *Menidia beryllina*. Then, using a population model, we found that the population-level impact of typical responses to EDCs, such as altered choriogenin production, strongly depend upon the shape of the mating function. Additionally, we found that masculinization is generally more detrimental to populations than feminization. We then used these results and the model to assess the status of wild silverside populations. Contrary to the expectation that a few males can spawn with many females, silversides exhibited a nearly linear mating function. This implies that small changes in the sex ratio due to EDCs will reduce reproductive success. For example, four out of five wild silverside populations simulated exhibited sex ratios far from 50:50 and thus are predicted to be experiencing population declines. Our results suggest that managers should place more emphasis on mitigating masculinizing rather than feminizing EDC effects. Another novelty of our approach is that our predictions are based on the reduction of choriogenin (protein level) following EDC exposure. In the context of adverse outcome pathways (AOPs), our model simulations directly link a key event (choriogenin production; downstream of a molecular initiating event) to a population-level outcome. Ultimately our results demonstrate that adverse outcomes can depend on population-level properties, such as the relationship between sex ratio and reproductive success.

Measurements and Methods in Environmental Nanotechnology in Aquatic Systems

MP111 Antimicrobial Effects of Silver Nanoparticles on the Microbial Community in a Freshwater Sediment from Sinking Creek

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Nanomaterials have been identified as environmental contaminants of concern, but their effects on biological systems are not fully understood. Silver nanoparticles (AgNPs) are the most commonly used nanomaterials in consumer products, due to their antimicrobial activity. The recent detection of AgNPs in municipal wastewater causes concern because of their potential risk to aquatic microbial communities, which provide important ecosystem services. Microorganisms living in the sediment have greater risk of exposure to AgNPs since the sediment serves as a sink for nanoparticles in aquatic systems. Evidence from previous studies showed the antimicrobial effects of AgNPs on microbial communities in marine estuarine sediments, but little is known about the AgNPs effects on microbial communities in freshwater sediment. This study evaluated the antimicrobial activity of citrate-coated AgNPs on the growth and activity of a freshwater sediment associated microbial community. Sediments from Sinking Creek were exposed to 1, 2, 4, 10, and 14 mg/L Ag in the AgNPs suspension (35 nm particle size) for 24 h. Subsamples of the exposed sediments were analyzed using heterotrophic plate counts and microbial enzyme activity assays for glucosidase and alkaline phosphatase to determine the antimicrobial effects of AgNPs. Preliminary results showed that low concentrations (1, 2, and 4 mg/L Ag) of the citrate-coated AgNPs stimulated increasing average growth of the microorganisms from 850 (±150) to 4447 (±25) CFU/gram, and then declined to 750 (±136) CFU/gram at the highest concentration (14 mg/L Ag). Further investigations showed citrate as the cause of the stimulated microbial growth at the low concentration of the nanoparticles, suggesting the use of the citrate as carbon source by the microorganisms for growth. The nanoparticles did not affect glucosidase and alkaline phosphatase activities. Our preliminary findings indicate that AgNPs affect the growth of microorganisms in freshwater sediment, as expected due to the demonstrated antimicrobial properties of AgNPs incorporated in commercial products.

MP113 Characterizing the Dissolution Kinetics of Nano-formulations of RDX and HMX

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The production of nano-organoenergetics formulations is a novel approach to increasing energetic yield and efficiency. However, there is concern that acquisition of this technology may be encumbered by a poor understanding if the particulate nature of these formulations will alter how fate and effects in the environment relative to traditional explosive formulations and residues. Of particular interest is the coating of the particles and how this may affect interactions in environmentally relevant media, dissolution kinetics, and ultimately biological interaction. The objective of this study was to determine the dissolution rates of nitrocellulose coated nano-HMX and nano-RDX and uncoated nano-HMX and nano-RDX. Materials were spiked into both ultrapure and moderately hard reconstituted water (MHRW) and sampled at various time points of 0, 4, 6, 24, 48 and 72 hours under static conditions. Nano-RDX was generally more stable with respect to suspension than Nano-HMX when tested at 300 ppm and 30 ppm in both ultrapure and MHRW. Nano-HMX was generally less stable than NanoRDX, with percent loss in total suspended concentration of 39% - 67% and 16% - 28%, respectively. It was determined that coated material exhibited slower dissolution rates than uncoated material. Dissolution of nano formulated munitions were similar to traditional HMX and RDX. This fundamental knowledge on nano-organoenergetics will help the Army characterize potential differences in fate and exposure in risk assessments.

MP114 Environmental Toxicity Assessment of Industrially Relevant Nanomaterials Using Bacteria Model

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An enormous growth in the use of nanotechnology-based products in the last ten years means that there is an increased risk of human and environmental exposure to engineered nanomaterials. Thus, it is important to investigate the fate and behavior of industrially relevant engineered nanomaterials in the environment, as well as their toxicity towards living organisms. Quantum dots are crystalline semiconductor nanoparticles with unique optical properties due to quantum confinement effects. They have some advantages over organic fluorescent dyes, such as high fluorescent brightness, photostability, and tunable emission wavelengths, dependent upon particle size. Their unique optical properties have led to increased use in a variety of devices, including diode lasers and television displays, as well as in biomedical research. The most commonly used quantum dots are cadmium selenide (CdSe) quantum dots, which contain inherently toxic cadmium. The first part of this work focuses on the comparison of toxic effects of conventional CdSe quantum dots and silicon quantum dots, which are emerging as a potential benign alternative, using bacteria as a model organism. The second part of the work explores the toxicity of copper zinc tin sulfide (CZTS) nanoparticles to the same bacteria. CZTS nanoparticles have potential applications as p-type absorber layer in solar cells for improving energy/fuel efficiency. The main bacteria model used for our studies is *Shewanella oneidensis* MR-1, which is a Gram-negative bacterium, mainly prevalent in deep sea anaerobic habitats, but can also be found in soil habitats. Some preliminary studies have been done on *Acinetobacter baylyi*, another Gram-negative bacteria, and *Bacillus subtilis*, a Gram-positive bacteria. This research assesses changes in cell viability, respiration pattern, and cell membrane integrity in presence of the nanoparticles. Results to date reveal that the silicon quantum dots are minimally toxic toward these environmentally critical bacteria, a significant improvement when compared to their cadmium-containing counterparts. In preliminary experiments, the CZTS nanoparticles do not pose any toxicity towards *Shewanella* cells, within a week of their synthesis, but an increasing trend in toxicity is observed over time.

MP115 Nanoparticle Composition, Morphology, and Reactivity Contribute to the Bacterial Toxicity of Nanoscale Metal-Oxides and -Phosphates

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The development of sustainable sources of power is a major societal endeavor. Safe and efficient storage of energy is tantamount to this effort, and lithium ion batteries (LIBs) are important tools to achieve this goal. One reason for the success of LIBs is the wide variety of cathode materials that allow for energy storage tailored to the needs of specific applications. These cathode materials are often heavy-metal based nanoscale materials, and as LIB manufacturing increases, the production of nanoscale cathode materials will occur on a larger scale. Although increased production, use, and disposal will lead to deposition of these nanomaterials into the environment, the interactions of these materials with organisms remains largely unexplored. Here, we study the interactions of a model environmental bacterium, *Shewanella oneidensis* MR-1, with two classes of LIB cathode materials: 1) nanoscale layered intercalation nanoparticles (NPs) with formula $LiNi_{0.33}Co_{0.33}Mn_{0.33}O_2$ (NMC); and 2) olivine-inspired NPs from the lithium cobalt phosphate and lithium iron phosphate families. Our groups previously demonstrated that nanoscale NMC exhibits a dose-dependent toxicity towards *S. oneidensis* MR-1 through the release of Ni and Co cations. Here, we study NMC with nano-sheet or nano-block morphologies and compare toxicities from these materials with that from a commercial microscale NMC. We find that material surface areas are correlated to the extent of heavy metal dissolution, which dictates the toxicity towards *S. oneidensis* MR-1. Separately, we studied the interactions of lithium iron phosphate and mulitiphase lithiated cobalt phosphate materials with *S. oneidensis* MR-1. Using two viability assays, we demonstrate that $LiFePO_4$ shows no toxicity towards this model organism at concentrations from 1–100 mg/L. At these same doses, lithiated cobalt phosphates exhibit significant toxicity with an LD_{50} of ~1 mg/L. Evidence suggests that solid-state aging of the lithiated cobalt phosphate NPs leads to decreases in toxicity, which we monitored over a several-month time course, accompanied by XPS and XRD characterization of the aged material. Our results indicate that particle composition, morphology, and reactivity all play significant roles in the environmental impact of nanoparticles relevant to electrochemical energy storage.

Development of Microbial Environmental Resistance

MP116 Analysis of Multiple Classes of Antimicrobials, PPCPs, and SSRIs in Wastewater and Surface Water from Samples Around the Globe

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Antimicrobial resistance is a serious threat because it is continuously emerging and spreading globally, reducing the potential of current treatment options to cure diseases. The release of antimicrobial drugs into the environment through effluents from wastewater treatment plants (WWTP) has been known to contribute to the development of antimicrobial resistance genes and bacteria. In order to contribute to an improved understanding of wastewater – mediated dissemination of antimicrobial resistance, the levels of antimicrobial drugs in WWTP systems from five different countries, Hong Kong, Philippines, Sweden, Switzerland, and the United States, were determined. A single LC/MS/MS method for the simultaneous analysis of 44 compounds from 6 different classes of compounds was used. Results showed that 26 out of these 44 compounds were detected in the WWTP samples, with the United States and Sweden having 21, Switzerland and Hong Kong having 20, and the Philippines, 15 compounds. In Sweden, Switzerland, and the United States, majority of the compounds that were found were SSRIs and macrolides. PPCPs and sulfonamides were most commonly found in the Philippines, and Hong Kong samples had mostly sulfonamides, PPCPs and SSRIs. The levels of antimicrobial drugs, PPCPs and SSRIs in influent, effluent, and surface water samples were successfully quantified using this sensitive and robust analytical method.

MP117 Antibiotic Resistance in Minnesota Lake Water and Sediment

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Despite the long natural history of antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARG), the relatively recent overuse and misuse of agricultural and pharmaceutical drugs has allowed for the selection of super-resistant bacteria as well as a myriad of ARGs. ARB and ARGs are increasingly viewed as environmental contaminants, posing a serious threat to public health due to the increasing ineffectiveness of antibiotics to combat microbial infections. In 2012, the Minnesota Pollution Control Agency (MPCA) sampled 50 randomly selected Minnesota lakes for 125 chemicals that included a wide variety of pharmaceuticals and other micropollutants. Ten antibiotics were detected, including fluoroquinolones, sulfonamides, trimethoprim, and carbadox in concentrations up to 275.94 ng/L. Lake water and sediment from 13 of these 50 lakes were analyzed for ARBs and ARGs. Bacterial antibiotic resistance was correlated to the level of antibiotics detected in the monitoring study. When plotted against the total antibiotics found in each lake, the correlation coefficient for the number of colony forming units (CFUs) grown on nutrient agar plates infused with 100 µg/mL of ampicillin was r=0.919. In addition, antibiotic resistant genes bla-1, SHV, TEM, sul1, sul2, and sul3 were also tested for in the lake samples. Seven of the 13 lakes were positive for TEM ARG, 6 of the 13 lakes for bla-1 ARG, while two lakes showed the presence of a sul ARG. These results suggest that an influx of low concentrations of antibiotics to lakes may be inducing microbial antibiotic resistance in these ecosystems.

Aquatic Toxicology and Ecology – Part 1

MP118 Assessing the lethal and sublethal toxicity of perfluorooctanoic acid (PFOA) to Hyalella azteca and Pimephales promelas

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Perfluoroalkyl substances (PFASs) are used in a variety of industrial and commercial products, including surfactants, polymers, lubricants, adhesives, paints, household cleaners, pesticides, and fire-fighting foams. Significant environmental concerns are associated with PFASs due to their persistence, potential for bioaccumulation, toxicity, and capacity for long-range transport. PFASs, including perfluorooctanoic acid (PFOA), are widely present in the Canadian environment, particularly at some contaminated sites due to historic firefighting training operations. Despite a large body of research on environmental exposure, the toxicity of PFOA is an emerging field of study, and insufficient aquatic toxicity data exist to develop water quality guidelines. Thus, our objective was to conduct chronic, aqueous exposures with PFOA to assess the lethal and sublethal toxicity to *Hyalella azteca* (amphipod) and *Pimephales promelas* (fathead minnow). Amphipod exposures were 6 weeks (1-100 mg/L nominal) and examined survival, growth, and reproduction. Fathead minnow exposures were 21 days (0.01 to 100 mg/L nominal), and endpoints included hatching success, deformities at hatch, larval survival, and growth. Measured PFOA concentrations in exposures were 80-90% nominal; therefore, toxicity data were expressed as nominal. Amphipod survival was significantly reduced at 100 mg/L, with a 6-week LC50 of 53 mg/L. Growth and reproduction of amphipods were more sensitive endpoints than survival, with 6-week EC50s of 2.3-2.4 mg/L. Fathead minnows were less sensitive than *Hyalella*, with only a 10% decrease in larval survival at 100 mg/L. There were some indications of increased deformities in larval fish at 100 mg/L, but these were not statistically significant. Hatching success and growth of larval fish were not affected by PFOA exposure up to 100 mg/L. Maximum concentrations of PFOA in the surface waters of the Great Lakes are generally < 50 ng/L, and as the toxicity of PFOA to amphipods and fathead minnows occurred at concentrations > 1 mg/L, it is likely that most environmental concentrations are far below those that cause toxicity to these species. However, localized areas could be highly contaminated due to historical activities or recent spills (where concentrations as high as 11 µg/L have been found). Our data will provide valuable information with which to assess the risk of PFOA at contaminated sites, and to set a target for site remediation.

MP119 Assessment of genotoxicity, oxidative stress and gene expression changes in zebrafish exposed to iron oxide (γ-Fe2O3) nanoparticles

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The increased use of nanotechnology in the last decade has raised concerns about the impact of nanoparticles in the environment. Particularly, the potential harmful effects of iron oxide nanoparticles (IONPs) in aquatic organisms have been poorly addressed. In this study, we used a multilevel approach to evaluate the acute toxicity of IONPs in adult zebrafish exposed for 96h to five sub-lethal concentrations (4.7, 9.3, 18.6, 37.2 and 74.4 mg/L). The approach included comet assay, micronucleus and nuclear abnormalities tests, thiobarbituric acid reactive substances (TBARS) assay and gene expression profiling (microarray). The genotoxicity assays performed in erythrocytes identified a significant number of DNA lesions in all IONP concentrations tested, compared to control. On the other hand, the TBARS test detected a significant small ~1.5-fold increase of malondialdehyde only in liver cells from organisms treated with the two higher IONP concentrations (37.2 and 74.4 mg/L), thus indicating mild oxidative stress at those concentrations. Microarray analysis of liver cells also exposed to the two higher IONP concentrations revealed 953 transcripts (927 genes) differentially expressed among the two treated groups and control samples. K-medoids clustering identified two gene clusters that separated treated samples from controls. Functional Analysis identified an enrichment of genes related to cation/metal ion binding, membrane formation and morphogenesis in Cluster 1 (over-regulated in treated groups); genes associated with RNA biogenesis, translation, ribosomes and several metabolic processes were identified in Cluster 2 (down-regulated in treated groups). Transcriptome data suggest that IONPs cause general negative effect on cell growth and a decreased ability of the cell to produce new proteins, although they do not induce a massive oxidative stress at higher concentrations. This is the first whole transcriptome report in an aquatic organism exposed to IONPs.

MP120 Assessment of Potential Amphibian Developmental Effects Following Field and Laboratory Exposures to Libby Amphibole Asbestos

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Sampling of Libby amphibole asbestos (LAA) in environmental media, including soil, water, and tissue, occurred at the Libby Asbestos Superfund Site, Operable Unit 3 (OU3), in Libby, Montana to support the preparation of an ecological risk assessment as part of a Remedial Investigation and Feasibility Study. This study was conducted in site and reference ponds to evaluate overall developmental health and assess the potential occurrence of histological lesions in amphibian species. Three target species of resident amphibians were collected: western toad (*Bufo boreas*), northern tree frog (*Pseudacris regilla*), and Columbia spotted frog (*Rana luteiventris*). A total of 315 specimens were collected from reference ponds and 477 specimens from Site ponds spanning a range of Gosner developmental stages. No malformations were identified in any of the larval (pre- and pro-metamorphosed) amphibians and only a single malformation was identified in one metamorph, subsequently attributed to predation. Statistical comparisons between site and reference specimens indicated some significant differences, but no consistent trends were identified between locations. Conclusions from the field assessment were that specimens from LAA-containing ponds and reference ponds were all normal and healthy appearing with variable growth and development patterns consistent with normal wild field amphibian populations. Metamorphosed tree and spotted frogs also underwent complete histopathology examinations. All observed lesions were attributed to causes other than LAA (e.g., inflammatory responses, parasitic lesions, or due to hypoxia). A laboratory study also was conducted to evaluate the effects of LAA in OU3 pond sediments on developing southern leopard frog (*R. sphenocephala*) larvae through metamorphosis. The median time to metamorphosis was 81.0 days for the control, 80.5 days for the reference sediment, and 82.0 days for the LAA sediment treatment. During the course of the study, no signs of overt toxicity were noted in any of the treatment groups. Additionally, no signs of asynchronous development, malformations or internal abnormalities were observed in organisms from the control, reference, or LAA sediment treatments. The conclusion of the laboratory study was that exposure of developing *R. sphenocephala* to LAA in sediment did not adversely affect the overall development or rate of metamorphosis.

MP121 Characterization of selenium uptake and trophic transfer through Canadian boreal lake food webs

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Selenium (Se) is increasingly being recognized as a contaminant of concern in aquatic systems, particularly due to its teratogenic effects on egg-laying vertebrates, but limited information is available on how Se moves through food webs in boreal lake systems. The objectives of the current research are to use limnocorrals (in situ enclosures) spiked with Se to characterize the uptake and trophic transfer of Se through a representative boreal lake food web. Nine limnocorrals (1 m depth, 2 m diameter) have been installed in the littoral zone of Lake 114 at the Experimental Lakes Area in Northwestern Ontario and were spiked with 1 µg/L or 10 µg/L Se (3 replicates each). These treatments will be compared to three controls with baseline levels of Se (approximately 0.15 µg/L). Biota will be collected biweekly to assess time to steady state and after 2.5 months of exposure, the uptake into periphyton, phytoplankton, invertebrates and female fathead minnows will be assessed. In addition, ecological (i.e. food web structure) and physiological (i.e. productivity and growth) factors affecting Se uptake by organisms will be assessed. Results of the above analyses will be discussed. This research will be important for risk assessment of selenium in Canadian boreal lakes, and for determining if current federal guidelines for Se are protective of these systems.

MP122 Correlating macroinvertebrate diversity and metal contamination in urban streams

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In recent years, there has been a more immediate need to understand the impacts of anthropogenic activities on freshwater systems. The streams along the Wasatch front in Salt Lake City, UT, act as a continuum in usage, access, and habitat type, all of which contributes to the amount, fate, and distribution of contaminants within the system. Interestingly, there is a relatively abrupt shift from rural to urban landscapes among all streams sampled. The objective of this research was to understand how endpoints, such as macroinvertebrate diversity and abundance, as well as water quality, differ within and among Wasatch front streams. Throughout the summer months of 2016 and 2017 City Creek, Emigration Creek, Parleys Creek, Mill Creek, Big and Little Cottonwood Creeks were surveyed. To survey macroinvertebrates passively, Hester Dendy artificial substrate samplers were deployed and allowed to colonize for 4 weeks, beginning in late May. Surber samplers were also employed as an active sampling method. Basic water quality data included temperature, pH, dissolved oxygen, and conductivity. We also analyzed for nutrient pollution (nitrate, ammonium, phosphate), and metals (copper, lead, cadmium). Preliminary analysis suggests that within all streams sampled headwaters (rural) and confluence (urban) transects have similarly low diversity, while mid-point transects, which were considered rural, had significantly higher diversity and abundance. Abiotic parameters, such as temperature and dissolved oxygen, increased and decreased, respectively, as streams became more urbanized. Because of the nonmonotonic nature of diversity and abundance, there was not a significant correlation between abiotic and biotic parameters. The highest concentration of nitrate to date (16.9 mg/L) was found in City Creek, one of the streams with the best access. Copper concentrations range from 0.03 mg/L in City Creek to 0.29 mg/L in Parleys Creek. Lead concentrations range from 0.113 mg/L in Big Cottonwood Creek to 0.445 mg/L in Little Cottonwood Creek. As the human population increases in urban areas, it is very important that we understand our impact on local natural resources. This study, and those like it, will aid future management decisions and help increase responsible public use practices.

MP123 Density dependence of copper algaecide exposures to Microcystis aeruginosa: Implications for microcystin-LR release

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Along with mechanistic models (e.g. Biotic Ligand Model), predictions of copper exposures and organism responses are often derived from laboratory toxicity experiments, which aim to standardize exposures and incubation conditions, providing the opportunity for comparison of inter- and intralaboratory toxicity data. For algae, cell density is an intrinsic exposure modifying factor that should be normalized among data, since cell density can alter the mass of copper sorbed per mass of algae and consequent population-level responses. These data are needed for pulse exposures of copper-based algaecides (used for mitigation of harmful algal blooms) and endotoxin (e.g. microcystin; MC) release. Laboratory toxicity experiments were conducted to physically model influences of *Microcystis aeruginosa* cell density on copper-algaecide (as copper ethanolamine) exposures and resulting algal responses in terms of MC release. The underlying hypothesis was that median effect concentrations (EC50s) for MC release should range among initial cell densities in terms of copper exposure concentration, but converge in terms of copper dose (sum of adsorbed and absorbed copper per unit mass of algae). Copper exposure concentrations were arrayed to result in a gradient of MC-LR release, and masses of copper sorbed to algal populations were measured following exposures. While copper exposure concentrations eliciting comparable MC-LR release ranged an order of magnitude (24-h EC50s 0.03-0.3 mg Cu/L) among cell densities of 10^6 through 10^7 cells/mL, copper doses (mg Cu/mg algae) were similar (24-h EC50s 0.005-0.006 mg Cu/mg algae). Comparisons of MC-LR release as a function of copper exposure concentrations and doses provided evidence of the density dependence of exposures in the context of copper-based algaecide applications. Measurements of exposure-response relationships for specific cell densities can serve as a link between laboratory and field scales for prediction purposes. These measurements can in turn decrease the likelihood of amending ineffective or excessive copper concentrations to aquatic systems, and minimize risks for non-target aquatic organisms.

MP124 Developing and Applying a Site-Specific Multimedia Fate Model to Address Ecological Risk of Oxytetracycline

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The overuse of oxytetracycline (OTC) in aquaculture has become a problem because of its chronic toxic effects on marine ecosystems. The present study assessed the ecological risk of OTC in the coastal waters near the Jangheung Flatfish Farm using a site-specific multimedia fate model to analyze exposure. Before the model was applied, its performance was validated by comparing it with field data. The coastal waters in the testbed were sampled and analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) followed by solid-phase extraction (SPE). The concentrations of OTC measured varied from 7.05-95.39 ng/L. The results of validating the models showed that the site-specific multimedia fate model performed better (root mean square error (RMSE): 24.217, index of agreement (IOA): 0.739) than conventional fugacity approaches. This result demonstrated the utility of this model in supporting effective future management of aquaculture effluent. The results of probabilistic risk assessment indicated that OTC from aquaculture effluent did not cause adverse effects, even in a maximum-use scenario.

MP125 Effect of hydraulic fracturing fluid on phototactic behaviour in Daphnia magna

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Hydraulic fracturing (HF) allows the improved extraction of gas or oil from the deep sedimentary deposits. HF involves injection of a solution of chemicals and proppants at high pressure, with the fluid brought back to the surface (termed Flowback and Produced Water; FPW) being a hypersaline solution containing metals, and both petrogenic and anthropogenic organic constituents. Acute toxicity studies elucidated a 48h-LC_{50} of 0.75% and 0.19% of total FPW, for adult and neonate *Daphnia magna* respectively, a key toxicity model species. Sublethal behavioural effects of FPW on *Daphnia* were then investigated. Positive phototaxis was inhibited in the presence of FPW in naively-exposed adults and neonates. Both adult and neonate daphnids were then acutely and chronically exposed to different concentrations of FPW before positive phototaxis was re-examined. Effects on behaviour were dependent on dose and length of exposure. This is the first study to investigate the impact of FPW on the behaviour of *D. magna*.

MP126 Effects of Exposing Fathead Minnows (Pimephales promelas) to a Known Estrogen Antagonist, Fulvestrant, During Sexual Development

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The objective of this study was to identify the effect of fulvestrant on fathead minnow larvae, and to then determine whether there were adult consequences to the larval exposure. We hypothesized that exposure to fulvestrant, a known anti-estrogen in mammals, during sexual differentiation would adversely impact sexual differentiation leading to a decrease in reproductive performance in adults. At 0 days post-hatch (dph), minnow larvae were randomly distributed among four groups: a control group, a solvent control (0.01% ethanol), a high dose group (2.29 µg/L), and a low dose group (0.03 µg/L). Exposure doses were based upon concentrations that elicited an adverse impact in fathead minnows in a previous study. Larvae were continuously exposed from 0-30 dph, covering the window of sexual differentiation. At the end of the exposure, relative expression of endocrine-responsive genes including estrogen receptors α and β, gonadal aromatase, vitellogenin, insulin-like growth factor 1, and androgen receptor were compared among the four treatment groups. Following the exposure, all fish were raised in clean water until sexual maturity at 135 dph. Adults were analyzed for morphometrics (i.e. gonadosomatic index, tubercle count, and relative ovipositor) and a 21-day reproductive assessment was performed on the remaining adults. At both doses, exposure to fulvestrant led to an upregulation in the expression of estrogen receptor β, vitellogenin, and androgen receptor, suggesting an estrogenic effect of fulvestrant. Sexually mature males in the fulvestrant exposed groups showed a reduction in gonadosomatic index and an increase in relative ovipositor length. These combined results suggest that fulvestrant, contrary to what we hypothesized and what is observed in mammals, is an agonist for fish estrogen receptors and has estrogenic effects on the development of the fathead minnow.

MP127 Evaluation of estrogenic activity in a demersal flatfish population near the municipal wastewater outfall of Orange County, California

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Wastewater treatment plant effluent often contains estrogenic agents, such as steroid hormones and certain industrial agents, which have been shown to induce estrogenic activity in wild fish populations. In the Southern California Bight, elevated plasma estradiol concentrations and estrogenic activity has been observed in male demersal flatfish and in sediments at the Orange County Sanitation District (OCSD) wastewater treatment plant outfall. However, the exact molecular and genetic pathways that are altered to induce these estrogenic effects in demersal flatfish and how they impact species at the population-level remain unclear. To select a species for further study, historic abundance and condition factor data of four flatfish species at the OCSD outfall were analyzed to determine whether populations were declining. Of the species examined, Pacific sanddab (Citharichthys sordidus) at the OCSD outfallshowed an 87% population decline from 2006 to 2017.However, condition index did not decline. To investigate whether population decline could be linked to adverse effects of estrogenic activity previously observed in other flatfish speciesin the area, livers of pacific sanddab from the outfall location and a reference site were evaluated for mRNA expression of vitellogenin, estrogen receptor alpha (ERα), estrogen receptor beta (ERβ), and CYP1A as well as measurements of [14C]-testosterone catabolism. In addition, plasma steroid hormone levels, and activities of estrogen biosynthetic enzymes,aromatase (CYP19A), 3-beta-hydroxysteroid dehydrogenase (3β-HSD) and 17-beta-hydroxysteroid dehydrogenase (17β-HSD) were measured in gonads. While estradiol levels in males were below the detection limit of 6.55 pg/mL, testosterone was detected but did not vary between outfall (T1) and reference (T11) sites. These preliminary data indicate that Pacific sanddabs did not have the same estrogenic responses as other demersal flatfish, such as Hornyhead turbot (Pleuronichthysverticalis) which did not have declining population. The results of this research will continue to explore potential mechanismsand causes for the declines in abundance of sanddabs at the outfall.

MP128 Evaluation of the Estrogen Receptor and the Dopamine Signaling Pathway as a Possible Target of Bifenthrin Toxicity in Zebrafish Embryos and Juveniles

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Bifenthrin (BF) is a pyrethroid insecticide widely used in urban and agricultural applications. Previous studies in juvenile fish have shown that environmentally relevant (ng/L) concentrations of BF can affect the endocrine system causing the over production of 17β-estradiol (E2) and altering the expression of dopaminergic pathway components in the central nervous system. We have also noted that BF acts as an anti-estrogenic compound in embryos but an estrogenic compound in juveniles. To examine the role of the estrogen receptor (ER) embryos were exposed for 96 hours to a mixture of 0.15 and 1.5 µg/L BF and an ER agonist (Ethinyl Estradiol – EE2). Likewise, juveniles were exposed for 96 hours to a mixture of 5µM E2 and two ER antagonists (ICI 182,780 and Raloxifene). Additionally, ER morpholinos (ERα, ERβ1, ERβ2) were injected in embryos and exposed to 0.15 and 1.5 µg/L BF and EE2. The relative levels of transcripts related to the dopaminergic and the hypothalamic-pituitary-gonad axis (DR1, and vitellogenin - VTG) were investigated by qRT-PCR, and dopamine and its metabolites (HVA and 3,4-dihydroxyphenylacetic acid) and E2 concentrations were evaluated by LC-MS/MS. Preliminary results show that BF decreased the estrogenic effects of EE2 in the embryonic stage, but in juveniles, ICI 182,780 and Raloxifene did not act as ER antagonists. These results show there are stage-dependent differences in the developmental toxicity of BF in zebrafish. In addition, the classic ER antagonists may not be effective in evaluating the role of ER in the toxicity of BF. Further analysis of differentially expressed genes coupled with endocrine responses will help assess potential sublethal targets of BF toxic and sub lethal effects using zebrafish as an animal model.

MP129 Examining Bioaccumulation of Select Contaminants of Emerging Concern in Clams, Mussels and Oysters

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By 2050 70% of human populations will reside in urban areas. High population density results in concentrated chemical use, which lead to exposures for human populations and ecosystems receiving waste streams within and from these urban centers. In developing nations, where many megacities will continue to emerge, access to chemical products is occurring faster than environmental management systems are being implemented. By 2050 it is estimated that global food production must increase by 50%. Aquaculture, which is growing 3-5 times faster than land-based agriculture, will play an important role to meet these needs. Unfortunately, 80% of the global sewage production is not treated, but returned to the environment and subject to reuse. These non-traditional waters are being recycled for agriculture, including aquaculture in peri-urban areas. We examined bioaccumulation of contaminants of emerging concern (CEC) in bivalves, primarily using isotope dilution LC-MS/MS. Additional analyses were performed for persistent organic pollutants and pesticides. Here, we report findings from our efforts in the USA and Hong Kong, where targeted analysis of CECs was performed in clams, mussels and oysters obtained from urban inland and coastal aquatic systems. For example, we examined CECs in green-lipped mussels and oysters collected adjacent to point source municipal wastewater and landfill leachate effluent discharges, respectively, in Hong Kong. We observed erythromycin, sertraline, carbamazepine, diphenhydramine and the drug of abuse ketamine above statistically determined MDLs at low µg/kg levels. These observations may support waste stream, water resource and food safety assessment and management in specific regions.

MP130 Export of Trace Elements by Emerging Dragonflies from a Wetland Constructed for Cu and Zn Effluent Treatment

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Dragonfly adults and their aquatic immature stages are an important part of food webs and provide a link between aquatic and terrestrial components. The H-02 wetland system was constructed on the Department of Energy's Savannah River Site, Aiken SC to treat building process effluents and stormwater runoff from a portion of H-Area. Constructed wetlands play an important role in the SRS environmental plan to achieve both federal and state regulatory compliance for the discharge of effluent waters. The H-02 wetlands were built to regulate pH and remove trace metals, primarily Cu and Zn, from the effluent line before release to state waters. The system consists of a retention basin, two wetland treatment cells, an effluent pool, and a discharge stream. Our previous work established contaminants accumulating in dragonfly nymphs throughout and below the wetland system. These studies have been expanded to assess contaminant export from the wetlands as dragonflies emerge from the water and enter terrestrial food webs. In addition to whole-body concentrations in teneral (emerging) dragonflies, we also analyzed exuviae (nymph exoskeleton) shed upon emergence. The latter indicates what proportion of the contaminant load accumulated by the nymph, which was primarily bound to the body surface and shed with the exuviae, compared to that actually leaving the wetlands in the emerging dragonflies. Dragonflies were collected from three sites in the retention basin. Traps were designed to sample both near-shore and pelagic habitats within each section. We collected and identified over 685 dragonflies and their corresponding exuviae to the species level. A total of 16 dragonfly species were collected that belonged to 2 families and 11 genera. Concentrations of 16 elements (Cu, Zn, Mg, Al, Fe, B, V, Cr, Mn, Co, Ni, Cd, Ba, Pb, Se, and As) were determined for each of over 400 individuals employing ICP-MS. Patterns of body burdens of emerging dragonflies and thus export from the system varied by genus and element. Species within a genus sometimes differed in accumulation. Section of the retention basin appeared to more influential on accumulation than habitat type within the section.

MP131 Gene transcription ontogeny of thyroid-axis development in early-life stage fathead minnows (Pimephales promelas)

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The hypothalamic-pituitary-thyroid (HPT) axis plays a critical role in regulation of metabolism, growth, and development in vertebrates. While the function of the HPT axis and thyroid hormone signaling in mammalian and amphibian development is well established, less is known relative to its role in fish. Baseline understanding of HPT-related mRNA expression may aid in predictions as to when early-life stage fish may be most susceptible to thyroid-disrupting compounds. In the present study, a time-course documenting transcription of key genes associated with the HPT axis over the course of early-life stages of fathead minnow (*Pimephales promelas*) development was evaluated. Fathead minnow embryos were sampled at eight time points between fertilization and hatch (5 days post-fertilization), and larvae were sampled approximately every other day, from hatch until 28 days post-hatch. Total RNA was extracted from pooled, whole fish, and mRNA transcription of thyroid-related genes was evaluated using quantitative polymerase chain reaction. Gene transcripts examined included: thyrotropin-releasing hormone receptor-2 (*trhr2*), thyroid stimulating hormone receptor (*tshr*), sodium-iodide symporter (*nis*), thyroid peroxidase (*tpo*), transthyretin (*ttr*), deiodinases 1 and 2 (*dio1* and *dio2*), and thyroid hormone receptors-alpha and -beta (*thra*and *β*). Baseline knowledge of thyroid-related mRNA transcription provided in the present study will aid in the development of adverse outcome pathways detailing impacts of thyroid axis disruption during fish early-life stages, and provide insights into the roles different components of the axis may play during fish development. The contents of this abstract neither constitute nor necessarily reflect USEPA policy.

MP132 Growth and physiological effects in Ankistrodesmus falcatus and Microcystis aeruginosa exposed to mixtures of Nickel and 2,4-D

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Aquatic organisms are exposed to complex mixtures of chemical pollutants. The interaction among them modifies the effect that as single substances produce. Nickel is a metal frequently used in the industry. Herbicides are widely used for weed control, being 2,4-D an auxinic herbicide commonly applied around the world. Phytoplankton is responsible for the production of oxygen and organic compounds through photosynthesis, playing an important role in trophic webs in aquatic environments. It is important to know how the mixture of commonly released chemical pollutant as Ni and 2,4-D affect primary producers. In the present study, the green microalgae *A. falcatus* and the toxigenic cyanobacteria *M. aeruginosa* were exposed to mixtures of different quantities of Ni^{2+} and 2,4-D that were previously determined as the equivalent to the inhibitory concentrations of IC_{10}, IC_{25}, and IC_{50}. Incubation conditions were continuous illumination and shaking, at 27 °C for 96 h. Cell density was daily quantified. At the end of the assay, protein, lipids, carbohydrates and photosynthetic pigments were determined. Also, observations with the scanning (SEM) and transmission electron microscopy (TEM) were done. For *M. aeruginosa* phycobiliproteins and cyanotoxins were quantified. *A. falcatus* exposed to the mixture of chemicals showed a significant decrease in cells density and increased in all the analyzed biomarkers; SEM and TEM images evidenced cell deformations. *M. aeruginosa* exposed to the mixture of chemicals diminished the cell density and increased the concentration of carotenoids, macromolecules, and cyanotoxins; additionally, ultrastructural alterations were observed with TEM analysis. Both organisms exposed to the mixture of Ni and 2,4-D present a higher inhibition of population growth, compared with data from both test organisms exposed separately to each toxicant. For the two phytoplankters, the tested nickel concentrations were lower than the limit established by the Mexican regulation as the allowable content in waste water discharges. Assayed 2,4-D quantities were higher than the environmentally relevant concentration; however, the results of the present study described the interaction among these two pollutants, that are of different chemical nature (organic and inorganic). Moreover, it could be expected that in eutrophic and chemically polluted environments containing mixtures of pollutants, cyanobacteria would be capable of increasing the production of cyanotoxins.

MP133 How much energy does the Great Pond snail Lymnaea stagnalis has and consumes when exposed to cadmium?

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The energy budget of an organism describes the distribution of energy to different organismal processes, mainly maintenance, growth and reproduction. Energy that remains after essential demands are met can be stored as energy reserves that could be later used if the organism is under stress. The purpose of this study was to study the effects of chronic cadmium (Cd) exposure on the energy metabolism of *Lymnaea stagnalis* and the effects of parental Cd exposure on their offspring. We exposed adult snails to Cd concentrations of 25, 50, 100 and 200 µg/L for eight weeks. Egg masses were collected at three different times during the eight weeks. After exposure, adult snails and all the egg masses collected were biochemically analyzed for total proteins, carbohydrates and lipids. Adult snails' lipid fraction was additionally separated into glycol-, neutral and phospholipids, and analyzed. The potential to process energy was estimated as a measurement of the electron transport system (ETS). Adult snails showed a significant increase in proteins and a significant decrease in carbohydrates after Cd exposure, while egg masses showed an increase in carbohydrates and a decrease in proteins with increasing Cd concentrations and longer exposure. Lipids did not vary with Cd exposure but the energy consumed (ETS) increased with increasing Cd concentration. This study provides evidence that energy either as available or consumed could be estimated and be used as an indicator of stress, specifically a contaminant exposure.

MP134 Hydraulic Fracturing Fluid Biocide, TTPC, Causes Mitochondrial Dysfunction That is Enhanced by NaCl in Chironomus Riparius

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One modern technology that currently concerns many environmental scientists is hydraulic fracturing. Hydraulic fracturing extracts oil and gas from deep underground and has expanded into areas where production was once considered impractical. Given the increase in hydraulic fracking, there is a risk that chemical components of fracturing fluids could enter fresh waterways and impact aquatic organisms. FracFocus disclosure lists additives used in fracturing fluid of 39,000 individual oil and gas production wells in the U.S.A. Additives include sodium chloride and tributyl tetradecyl phosphonium chloride (TTPC) as a biocide to minimize bacterial contamination of hydrocarbons. Waste water produced from hydraulic fracturing can contain total dissolved solids and Cl exceeding 100,000mg/L. According to HALLIBURTON company, the concentration of TTPC in fracturing fluid is 300 mg/L. In this study, the acute toxicity of TTPC and NaCl were tested using 4th instar *Chironomus riparius*. Results showed that 48 h LC50 of TTPC and NaCl were 0.48mg/L and 9368mg/L, respectively. As a part of investigating mechanisms of action of TTPC, ATP was measured from hemolymph samples of larvae raised in various concentrations and collected at 24h. Results showed that ATP levels increased at low concentrations then decreased as concentrations and concurrent mortality increased. This likely demonstrated an initial defensive response followed by a loss in mitochondrial membrane integrity. Red (cool) laser technology was used to investigate the integrity of mitochondria. ATP levels increased at low TTPC concentrations when larva were exposed to the laser. This indicated that mitochondria were still functioning, but as the mortality increased, excitation of red laser did not stimulate additional ATP production demonstrating injury to the mitochondria. These studies should provide information about the sensitivity of aquatic biota to components of hydraulic fracturing as well as toxic mechanisms of action that can help distinguish effects of TTPC from other environmental stressors.

MP135 Identification of toxicity pathways that can be used to predict adverse outcomes of chlorpyrifos in fathead minnows

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Chlorpyrifos is an organophosphate insecticide that acts as a neurotoxicant through inhibition of the enzyme cholinesterase. The mode of action of organophosphates in target and non-target organisms, including mammals, is similar. The aim of the project is to develop an early life-stage gene expression assay (EcoToxChip) that captures critical toxicity pathways of chlorpyrifos for the prediction of apical outcomes of regulatory relevance. As this assay is intended to use early life-stages that are not feeding independently, it would not be considered as a live animal test, and therefore, would address the need for alternative approaches in chemical screening. As part of the project, critical toxicity pathways and associated core genes will be identified following exposure of fathead minnows (*Pimephales promelas*) at early life-stages to three sub-lethal concentrations of chlorpyrifos. Specifically, sequence-by-synthesis-based whole transcriptome (RNASeq) and high-resolution mass-spectrometry-based shotgun proteomics will be used to characterize key molecular toxicity pathways. Pathways will then be correlated with downstream biological responses of ecological and regulatory relevance, and critical genes linked to apical outcomes will be identified for inclusion on EcoToxChips. Chlorpyrifos concentrations were selected based on a preliminary test as well as concentrations in published data. These tests revealed a threshold level of mortality between 1 and 10 µg/L chlorpyrifos. To ensure the determination of solely sub-lethal effects in at least two of the tested concentrations, 0.5, 1.5 and 4.5 µg/L chlorpyrifos solutions were investigated in the fathead minnow early life-stage assay with larvae samplings after 7 and 32 days of exposure. None of these concentrations affected survival or growth, resulting in a sub-chronic NOAEC and LOAEC of 4.5 and 10 µg/L chlorpyrifos, respectively, in fathead minnows. Samples are currently being further analyzed for molecular and physiological endpoints to gain insight into critical toxicity pathways. This study is part of the EcoToxChip project (@ecotoxchip).

MP136 Impact of sediment-associated uptake on the food web transfer of organic pollutants between pelagic and benthic food webs in eutrophic Lake Dianchi

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Trophic transfer of organic pollutants in aquatic ecosystems is an important criterion for assessing their ecological risk. This study analyzed various organic pollutants in the pelagic and benthic food webs of Lake Dianchi, and found that the p,p'-DDE, and PAH compounds (including PAHs and substituted-PAHs) showed significantly different trophodynamics between pelagic and benthic food webs. The TMFs of p,p'-DDE, and PAH compounds were calculated to be 3.33 and 0.14-0.38 in pelagic food web, and 2.07 and 0.73-0.94 in benthic food web, respectively. Concentrations of all pollutants exhibited lower specie-differences in benthos compared to pelagic organisms due to extra uptake via ingested sediment in benthos. The estimated uptake proportions via sediment of trophic-dilution PAH compounds (0.66-0.71) were higher than those of trophic-magnification p,p'-DDE and CH_3Hg (0.35-0.45). Great habitat differences between pelagic and benthic organisms due to eutrophication of lakes would result in the different uptake proportions via sediment and subsequently different trophodynamics of pollutants in the two types of food webs.

MP137 Increased Temperature Alters Toxicity of Dietary Selenium to Larval Lithobates sphenocephalus and Hyla chrysoscelis

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With worldwide amphibian population declines, there has been an increased scientific interest in evaluating multiple stressors that could contribute to reduced populations. As amphibians are ectotherms, their metabolic rates increase with temperature within the critical thermal range. With global temperatures rising, it is important to evaluate how temperature-induced metabolic increases will alter the response to bioaccumulative contaminants. While for some contaminants there is a positive correlation between toxicity and temperature, increased metabolic activity due to elevated temperature might also lead to increased rates of excretion or metabolism of contaminants. Selenium (Se) is a ubiquitous contaminant in areas affected by fossil fuel combustion and processing where it is often the dominant compound in the mix of effluents that is accumulated and retained by larval amphibians. To address the influence of temperature on the toxicity of dietary selenium to these animals, I conducted a factorial experiment with two species of larval amphibians common to southern Maryland, Cope's grey tree frogs ("GTF" -*Hyla chrysoscelis*) and southern leopard frogs ("SLF" -*Lithobates sphenocephalus*). Temperature (22 or 29 C) and food-borne concentration of seleno-L-methionine (0ug/g- "control", ~13ug/g- "low," ~75ug/g-"high"), based upon food-borne concentrations measured in a contaminated site, were the manipulated variables. For SLF, survival was lower across all food concentrations at high temperature, with a compounding effect of dietary Se. I observed 100% mortality in both low and high Se concentrations at 29 C before metamorphosis was reached. Metabolic rate, measured as respiration rate, was quantified twice during larval development and following metamorphosis. Studies of GTF are underway. When complete, this project will establish links between selenium exposure and thermal metabolic physiology and therefore will represent a step forward in understanding chemical toxicology in the context of a changing thermal climate.

MP138 Is a Frog a Fish with Lungs? A Case Study of the Fungicide Trifloxystrobin

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Current risk assessment practice is to use fish as a surrogate for larval stage amphibian species in determining chemical sensitivity when data for amphibians is not available. While evidence exists that fish are a sufficiently sensitive surrogate for amphibians for a wide range of chemicals, there may be cases where this approach is inadequate. Of important consideration is the drastic life history differences between model fish species and amphibians which must undergo the complex process of metamorphosis. As pesticide exposure has been suggested to contribute to amphibian population decline it is important to determine if this form of surrogacy in risk assessment evaluations is protective of amphibian populations. In this study, zebrafish (*Danio rerio*) and African clawed-frog (*Xenopus laevis*) embryos (< 24 hpf) were exposed to 0 – 200 µg/L trifloxystrobin (0.005 % acetone) for 10 days in a static renewal system. Both species were monitored daily for mortality, developmental abnormalities, hatching and pigmentation. On day 10, individuals were weighed, photographed for length measurements, and flash frozen for future gene expression studies. Log-logistic 96-hr LC50 curves for *D. rerio* and *X. laevis* show that *X. laevis* is more sensitive to trifloxystrobin exposure than *D. rerio* with LC50 values of 45.85 ± 3.98 µg/L and 135.86 ± 7.58 µg/L, respectively. While a delay in hatching and pigmentation was observed in *D.rerio* no effects on growth were observed. Conversely, a dose-dependent decrease in weight and a delay of development were observed in *X. laevis* as fewer trifloxystrobin tadpoles reached NF stage 50 by the experiment termination than control tadpoles. Tissues collected from this study will be used to elucidate a molecular basis for species sensitivity comparison by assessing differential gene expression and guiding future comparative toxicology studies.

MP139 Mercury Bioaccumulation and Biomagnification in Headwater Streams of Tennessee's Appalachian Mountain

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While the Appalachian Mountains of Tennessee are often thought of as pristine due to their isolation from known point-source contaminants, their elevation makes them susceptible to high levels of contaminant deposition. In particular, mercury deposition has been measured at concentrations >10 mg/m^2/year, raising potential concerns for human and wildlife health. Tennessee's Ecologically At-Risk Streams – Appalachian Mountain (TEARS-AM) is a three-year study designed to determine the variability across and the susceptibility within four naturally-reproducing eastern brook trout (S*alvelinus fontinalis*) streams spanning the latitudinal gradient of Tennessee's Appalachian Mountains. To determine mercury exposure at these sites, eastern brook trout were collected and analyzed for mercury during the summer of 2015 along with mercury in water. Although the average concentration of mercury in water was 0.0001 PPB, the maximum concentration of mercury in whole-body fish homogenates (97 PPB) approached threshold values for piscivorous mammals (100 PPB). To better understand the bioaccumulation and biomagnification of mercury in headwater streams, the following year additional aquatic biota were collected alongside eastern brook trout and analyzed for mercury and stable isotopes (δ^{13}C and δ^{15}N). Presented here are the results of mercury in water and biota from 2015 and 2016.

MP140 Neurobehavioral effects of neonicotinoids on embryo-larval zebrafish at environmentally relevant concentrations

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Neonicotinoids are common surface water contaminants in both urban and agricultural landscapes. Mean stream concentrations in the U.S. were recently reported at 175 ng/L and 66 ng/L for imidacloprid and clothianidin, respectively (Bradley et al., 2017). Neurobehavioral effects on larval fish are known to occur at concentrations higher than those reported; however, behavioral effects have yet to be investigated at environmentally relevant concentrations. For this ongoing study, the developmental neurobehavioral effects of neonicotinoid exposure on embryo-larval zebrafish (*Danio rerio*) were assessed using a larval zebrafish behavior assay. Three of the most common neonicotinoids (clothianidin, imidacloprid, thiamethoxam) and two environmental imidacloprid metabolites (desnitro-imidacloprid and 6-chloronicotinic acid) were assessed individually and as part of environmentally relevant mixtures. Embryos were exposed to neonicotinoids at 1000, 100, 10, 1, 0.1, 0.01 µg/L starting at 4 h post-fertilization (hpf) to 5 d post-fertilization (dfp). Behavior assays assessing swimming behavior (distance traveled and velocity) were conducted at 5 dpf. Data were analyzed using one-way ANOVA with Tukey HSD post hoc test. Zebrafish larvae traveled a significantly greater distance at 1000 µg/L Imidacloprid ($p < 0.05$) compared to 100, 10, 0.1, 0.01 µg/L. All treatment groups travelled greater distance than the control group. Velocity of larva exposed to 1000, 100, 1 µg/L imidacloprid were significantly higher than those in the lowest treatment group 0.01 µg/L and control group. The results for the other neonicotinoids, imidacloprid metabolites, and mixtures are forthcoming; however, the preliminary imidacloprid data indicates that larval exposure to neonicotinoids causes behavioral changes at 1000 µg/L and may also cause behavioral changes at lower, more environmentally relevant concentrations.

MP141 Paraquat alters mitochondrial bioenergetics, locomotion, and the expression of the dopamine system in zebrafish (Danio rerio) larvae

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Paraquat is a dipyridyl herbicide that causes oxidative stress in cells and is implicated in neurodegenerative disease. In this study, zebrafish (Danio rerio) embryos at 6 hours post fertilization (hpf) were exposed to 1, 10 and 100 µM paraquat for 96 hours to learn more about the mechanisms underlying paraquat toxicity. Paraquat did not induce significant mortality nor deformity in developing fish up to 100 µM, but it did significantly accelerate time to hatch at all three doses examined. To determine if ATP production was related to changes in hatch rates, oxygen consumption rate was measured in whole embryos after a 24-hour and 48-hour exposure. Maximal respiration of embryos at 24 hpf was decreased at the highest concentration, suggesting that paraquat negatively affects oxidative respiration, but this effect was time dependent and there were no changes in the oxygen consumption rate of embryos at 48 hours. Due to evidence of impaired mitochondrial function, transcriptional responses of stress- and apoptosis-related genes were measured. Fish in 1 µM paraquat showed significantly higher expression levels of superoxide dismutase 2 (sod2), heat shock protein 70 (hsp70), bax, and B-cell CLL/lymphoma 2a (bcl2) compared to control fish, but there were no differences in transcript levels for larvae in the 10 and 100 µM paraquat treatments compared to control fish. This non-monotonic dose response was observed for a number of transcripts, which implies that the response in zebrafish larvae may be more pronounced at lower doses of paraquat. We also measured larval behavior at 7 dpf, and locomotor activity was stimulated with 100 µM paraquat. As dopamine is known to regulate behavior, and dopamine neurons are known to be primary targets for paraquat neurotoxicity, we investigated the effects of paraquat on the expression of the dopaminergic system as a putative mechanism underlying behavioral changes to paraquat. No difference in transcript levels were detected among groups for dopamine synthesis, but we did observe increased expression of the dopamine transporter (dat) and dopamine receptor D3 (drd3) in zebrafish exposed to 1 µM paraquat, suggesting a compensatory mechanism in dopaminergic signaling of larvae at low doses. This study reports on the underling molecular mechanisms associated with altered behavior in zebrafish, improving understanding into the toxic actions of this herbicide.

MP142 Pollutant-Induced Changes in RyR and CaV1 Alter DREAM-Mediated Gene Transcription

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Several environmental pollutants, including polychlorinated biphenyl (PCB) congeners and triclosan, are capable of causing Ca^{2+} signal disruption (CSD) by altering the activity of the ryanodine receptor (RyR) or the L-type voltage gated Ca^{2+} channels (CaV1). These two channels are important to countless physiological processes, but the extent to which CSD through these channels contributes to altered cellular pathways is currently unclear. We investigated whether CSD, caused by cellular exposure to PCBs and triclosan, can cause changes in gene transcription as regulated by the Ca^{2+} -sensitive transcriptional repressor DREAM (downstream regulator element antagonistic modulator). This research utilized the GT1-7 hypothalamic neuronal cell line and the TαT1 thyrotrophic cell line to measure whether CSD alters transcription of gonadotropin releasing hormone (GnRH) or thyroid-stimulating hormone (TSH), respectively. Cells were exposed to varying concentrations of each pollutant for multiple time periods, and GnRH and TSH levels assessed using qPCR. GT1-7 cell exposures to the potent RyR activator PCB 95 did not lead to changes in GnRH mRNA expression, which was supported by low RyR basal gene expression in the cell line. Exposure of GT1-7 cells to triclosan decreased GnRH transcription in a dose-dependent manner. Triclosan is known to inhibit CaV1 channels leading to decreased Ca^{2+} entry into the cell. When intracellular Ca^{2+} concentrations are decreased, DREAM remains bound to DNA, repressing transcription. DREAM is important to proper functionality of the digestive system, central nervous system, and skeletal and cardiac muscle, and it has been tied to pain reception, learning and memory and thyroid-gland health. This work will help address whether CSD is contributing to such alterations by altering DREAM-mediated transcription.

MP143 Quantitative Survey of Freshwater Mussels (Unionoidea) and Assessment of Sediment Contamination in the Old Lead Belt, Big River, Southeast Missouri

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Mining and mill waste in the Old Lead Belt of Southeast Missouri has contaminated over 150 km of sediment in the Big River over the Probable Effects Concentration (PEC) for Pb (MacDonald et al. 2000). Previous studies of freshwater mussel distribution (Roberts et al. 2009) and toxicity to juvenile mussels (Besser et al. 2008) revealed a good relationship between laboratory toxicity and mussel community metrics. However, mussel community metrics were depressed at lower concentrations of heavy metals than would be predicted by laboratory toxicity. Habitat factors, such as sediment embeddedness, were cited as a possible explanatory variable for reduced mussel densities where sediment toxicity was not predicted in the laboratory (Roberts et al. 2009). In 2013-2014, the relationship between freshwater mussel density and heavy metal concentrations in river sediments was investigated in the Big River with the goal toward discerning contributions of habitat factors versus sediment toxicity as explanatory variables. Quantitative mussel surveys were conducted and river sediments were analyzed for grain size and concentrations of Pb, Cd, and Zn at 18 sites spanning 120 km of river. Tissue samples were also collected of Asian clams (*Corbicula fluminea*) to determine body burdens of heavy metals and verify exposure of bivalves. Mussel density negatively corresponded to elevated concentrations of sediment Pb downstream of mining operations. A significant decrease in mussel density was observed downstream of mining sites where Pb concentrations were greater than the PEC. As Pb concentrations decreased to near the PEC, mussel density increased at most sites. While mussel densities recovered at RK 47, this did not correspond with a recovery of mussel richness until RK 16.5 where the mussel fauna is comparable to reference streams. *Corbicula fluminea* tissue Pb concentrations were correlated with mussel density and sediment Pb concentrations. Lead concentrations in *C. fluminea* tissue correlated with Pb in sediment and mussel density more strongly than similar comparisons between Zn and Cd concentrations in tissues. Mussel population metrics were not correlated with sediment grain size and other substrate metrics. Comparisons of mussel species diversity using available data from other similar rivers in Missouri indicated a 70 to 75 percent decline of mussels in Pb contaminated areas of the Big River.

MP144 Relationship Between Oil and Toxicity in Newtown Creek Sediments

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This study explores the relationship between oil contamination in Newtown Creek and sediment toxicity (expressed as either ten day or 28 day survival of L. plumulosus) using simple regressions of contaminant concentrations and survival. The underlying assumption is that the presence of oil has a clear physical effect on the survival of the test organisms that confounds any dose response between bulk sediment and pore water concentrations of various individual chemical contaminants. The toxicity data from Newtown Creek displayed a bimodal distribution with certain upstream reaches of the creek exhibiting very low ten and 28 day survival and the main stem of the creek exhibiting generally higher survival. Three sets of observations indicate that there appears to be a physical effect of oil on the toxicity test results: (1) the general, although not universal, spatial correlations between the visible presence of oil in test sediments and very low (generally less than 10% survival in either test); (2) the occurrence of generally higher quantitative measures Total Petroleum Hydrocarbons and Diesel Range Organics among those test samples taken from the areas of lower survival; and (3) A reasonable regression between TPH or DRO and survival in both tests. Specifically: There appears to be a spatial correlation between toxicity test sediments that have visual evidence of oil and the measurement of extremely low survival in both the ten day and 28 day tests; Quantitative measurements of oil demonstrate that TPH concentrations are highest (averaging above 5,000 ppm) in the creek Turning Basin and all tributaries (including East Branch and Dutch Kills where there were no observations of visual evidence of oil); Quantitative measures of oil in sediments (either expressed as TPH or diesel range organics) have close correlations (R-squared ranging from 0.466 to 0.8297); The closest correlation (R-squared 0.8297) was between diesel range organics (DRO) and 28 day survival; These correlations allow the estimate of an oil based preliminary remediation goal (as a no observed effect level) that is consistent with no effect levels associated with bulk oil exposures and toxicity in the literature; The application of an oil based preliminary remediation goal would also result in acceptable bulk sediment and pore water concentrations in Newtown Creek (based on triad samples only at this time).

MP145 Reproductive effects of early life stage thyroid disruption in the fathead minnow

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Exposure to contaminants during development has the potential to cause adverse biological alterations that can persist through depuration periods and into adulthood. This study examined the effects of chemically induced, early-life-stage thyroid disruption on endpoints associated with thyroidal and reproductive function in the fathead minnow (*Pimephales promelas*). Fish were exposed to propylthiouracil (PTU) from 1 to 43 days post hatch (dph) to induce hypothyroidism. At the end of exposure, length and weight were measured and samples were taken for gene expression analysis. The remaining fish were transferred to un-dosed water and raised to maturity and, at 164 dph, a 21-day breeding assay was performed. At the end of exposure, fish exposed to PTU had significantly reduced length and weight indicating successful thyroid disruption. There were also significant differences in expression of several genes involved in the thyroidal and reproductive signaling systems. After maturation, there were no significant differences in any morphological variables. During the 21-day breeding assay, fish from the PTU exposure group had significantly reduced overall fecundity relative to controls. Based on data collected so far, it appears that this reduction in fecundity is due to either ovarian dysfunction or alterations in reproductive behavior. The results show that early-life-stage hypothyroidism can affect reproductive function later in life even after thyroid related endpoints have returned to control levels.

MP146 Selection of housekeeping genes for the biomonitoring of gene expression in Gambusia yucatana in Yucatan, Mexico

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In Yucatan's peninsula, groundwater is the main water supply for human, agricultural and industrial activities. Ground characteristics make recovery and treatment of wastewater very expensive. This situation has contributed to an increase of pollutants reports in Yucatan's peninsula aquifer. Unfortunately, studies related to the effect of those pollutants in native organisms are scarce. Expression of genes CYP1A, metallothionein (MT) and vitellogenin (VTG) using qRT-PCR have been widely used to study the effect of pollutants in biomonitoring studies. The estimation of gene expression at the mRNA level requires methods for accurate normalization. Control genes, or housekeeping genes are used to normalize expression between samples. An ideal housekeeping gene should have stable tissue expression, and should not change under any circumstance. In real life, the ideal housekeeping gene doesn't exist. Thus, the selection of housekeeping genes is critical for gene expression studies and should be carefully evaluated. The aim of this study was to evaluate the use of β-actin, elongation factor 1α (E1Fα) and ribosomal protein L7 (RPL7) as housekeeping genes to be used to normalize expression in male and female *G.yucatana*. Analysis of the stability of the tested housekeeping genes indicated that in females the lowest standard deviation of genes was β-actin > RPL7 > E1Fα and in males was E1Fα > β-actin > RPL7. The highest coefficient of variation was found in E1Fα and RPL7 in females and males respectively. Pearson's correlation analysis indicated very high correlation (above 0.8) between these genes, therefore a method using the 3 housekeeping genes and their efficiencies were used to evaluate the expression on CYP1A, MT and VTG in wild mosquitofish. The analysis of *G. yucatana* samples from seven "cenotes" (sinkholes) indicated that some of them presented significant differences from control mosquitofish for CYP1A, MT and VTG in male and female mosquitofish. Cenotes used for recreation or near farms where the ones in which mosquitofish genes were upregulated. These results constitute an early warning that evidence the need to promote programs to monitor pollutants and their effects in aquatic organisms in Yucatan's groundwater. This study was financed by grant UNAM-DGAPA-PAPIIT #IA202416 and by the Faculty of Chemistry PAIP program.

MP147 Sublethal TCDD Exposure During Zebrafish Development Produces Multigenerational Testicular Abnormalities in Histology and Gene Expression

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The industrial by-product TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) is a potent environmental toxicant and endocrine-disrupting chemical (EDC) with known multigenerational teratogenic effects on humans, rodents and fish. A developmental basis of adult-onset diseases has been implicated following exposure to some EDCs. Zebrafish (Danio rerio) are effective in modelling developmental and multigenerational toxic effects of EDCs, due to their short generation time, transparency in early development, and ease of developmental exposure. Previous work in this lab has shown that both structural and reproductive abnormalities (spinal deformities, sex ratio skewed toward female fish, body plan/gonad mismatch, and decreased fertility) were observed in young zebrafish exposed to TCDD. Reproductive abnormalities observed in subsequent unexposed generations (F1 and F2) were male-mediated, suggesting transmission through the male germline. We analyzed the testicular tissue of TCDD-exposed male zebrafish from all three generations, looking for changes in histology and gene expression that could account for decreased reproductive capacity. For histological analysis, spermatogenic cells were categorized by differentiation stage and quantified within seminiferous tubules. Statistical analysis demonstrated significant differences in certain spermatogenic cell types between exposed and control groups in the F0 and F1 generations, indicating delayed spermiation in exposed males and their offspring. Gene expression analysis of testis samples revealed altered expression in spermatogenesis, steroidogenesis, lipid metabolism, and aryl hydrocarbon receptor (AhR) xenobiotic response pathways in exposed fish in all generations. Overall, differential expression of reproductive genes and reduced capacity of sperm cells to mature could account for the reproductive defects previously seen in TCDD-exposed male zebrafish and their descendants.

MP148 The Combined Effects of Atrazine and Tetracycline on Primary Producers and Zooplankton in Freshwater Microcosms

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Widespread use of agrochemicals such as herbicides and antibiotics increases their likelihood of entering aquatic systems in mixture. Despite different modes of action, atrazine (herbicide) and tetracycline (antibiotic) each adversely affect non-target photosynthetic organisms, including algae and macrophytes, with the potential to reduce food availability to higher trophic levels. However, to date the effect of simultaneous exposure to both contaminants on aquatic communities has not been determined. We hypothesized that a mixture of atrazine and tetracycline affects freshwater communities differently than each compound alone. A green microalga (*Chlorella* sp.), a duckweed species (*Lemna minor*), and a crustacean zooplankter (*Daphnia magna*) were exposed to an environmentally relevant concentration of atrazine, tetracycline, or both in greenhouse microcosms for 12 days. The endpoints measured were *Chlorella* sp. cell density and chlorophyll *a* concentration, *L. minor* abundance and growth inhibition, and *D. magna* mortality and reproduction. Cell density was unaffected, but atrazine alone reduced *Chlorella* sp. chlorophyll *a* concentration. Abundance of *L. minor* declined in the presence of tetracycline, trended toward a decrease due to atrazine exposure, and the adverse effects of both atrazine and tetracycline on abundance appeared additive. Growth inhibition of *L. minor* also appeared additive, with 33% inhibition in the presence of atrazine, 29% following tetracycline exposure and 69% in the combination treatment. Mortality and reproduction of *D. magna* were unaffected by any of the treatments. Overall, *L. minor* experienced the most damage in mixture compared to each contaminant alone. In contrast, *Chlorella* sp. exhibited fewer adverse responses to treatments than expected, which may in part be due to contaminant removal from the water column by *L. minor*. No evidence of direct toxicity to or food limitation of *D. magna* was observed, but decreased *Chlorella* sp. chlorophyll *a* concentration following atrazine exposure could reduce food resource quality over longer time periods. These findings highlight the importance of examining community-level responses to environmentally relevant concentrations of contaminant mixtures to aid water resource management.

MP149 The combined effects of cadmium and glyphosate on the life history of Daphnia magna

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Ecological risk assessment methods have generally focused on single stressor exposure scenarios. However, rarely do data from single stressor studies provide enough predictive power to understand the effects of combined stressors, because effects may be greater than additive, and interactions are likely to result in nonlinear responses. Specifically, exposure to multiple stressors, such contaminant mixtures, can produce adverse effects that are greater than those of either individual stressor. The toxicity of metal and herbicides on sublethal endpoints is of particular interest among toxicologists today. Cadmium is ubiquitous in most environments and is known to be a model contaminant in laboratory studies. Glyphosate is the second most used herbicide on crop lands in the state of Utah. The objective of the present study is to characterize the interactive effects of the combined stress of cadmium and glyphosate on life history endpoints of Daphnia magna. Individual daphnia will be exposed in a full factorial design to concentrations of cadmium (0.5 and 2 µg/L) and glyphosate (50 and 450 µg/L). Life history will be observed during a 21-day assay. Endpoints include time to first reproduction, number of offspring per broods, and number of total broods. To understand whether stressors cause additive or greater-than-additive effects in combination, we employed the independent action model. Preliminary data of the cadmium treatments suggests that D. magna exposed to cadmium show increased time to first reproduction and decreased output for both high and low treatment groups when compared to control. Within glyphosate treatments, reproductive endpoints are not different from controls. Additionally, mixture treatments seem to have greater-than-additive effects on D. magna reproduction. This may be a result of potentiation in the mixture treatments. It is our hope that these data will help improve our understanding of mixture toxicology and the effects of contaminants on sublethal endpoints.

MP150 The effect of carbaryl exposure on the metabolic rate of larval amphibians

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Metabolism governs the energy budget and resulting resource allocation in living organisms. When individuals are forced to allocate energy to processes managing chemically-induced tissue damage or detoxification, tradeoffs are made that have consequences for the energy budget as a whole. Larval amphibians are commonly subject to chemical exposure in their aquatic environments via agricultural runoff, and are thus a relevant system in which to investigate these energetic tradeoffs. Here, we exposed four species of larval amphibians to a chemical-free control or one of three environmentally-relevant concentrations of the insecticide carbaryl: 0.5 ppm, 2 ppm, and 4 ppm. The two lower concentrations were administered daily over the course of two weeks as chronic exposures, while the highest was administered 24 hours prior to testing as an acute exposure. We used a closed-system aquatic respirometer to calculate oxygen consumption as a proxy for metabolic rate. We found a significant decrease in oxygen consumption by those individuals treated acutely with carbaryl as compared to controls, though the chronic doses did not significantly alter oxygen consumption. In wood and leopard frogs, acutely exposed individuals consumed 28.2% and 20.8% less oxygen respectively relative to controls, a trend that appeared to be largely driven by behavior. Lethargy reflective of a decreased metabolic rate may result in reduced foraging or ability to defend against other stressors. Further research is necessary to examine these energetic tradeoffs associated with chemical contamination in aquatic systems.

MP151 The effects of hydroxypropyl-β-cyclodextrin (HPβCD) on the toxicity of intraperitoneally injected steroids in rainbow trout (Oncorhynchus mykiss)

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The risk of pharmaceuticals and personal care products (PPCPs) in the aquatic environment has been heavily studied in recent years. Much focus of this research has involved individual compounds. While this information is essential for understanding and highlighting individual toxicity, it does not take into consideration the toxicity of chemicals in a mixture. This is a concern since PPCPs most commonly enter surface waters through wastewater treatment plant (WWTP) effluent as a complex mixture. This study investigated how the odour suppressant and excipient hydroxypropyl-β-cyclodextrin (HPβCD) alters the toxicity of select synthetic steroids commonly used as human oral contraceptives. HPβCD is both used as an excipient in the pharmaceutical industry as well as an odour suppressant in Febreze®. HPβCD is amphiphilic and toroidal in shape with the ability to include non-polar compounds within its central cavity, including many synthetic steroids. The steroids of interest include: 17α-ethinylestradiol (EE2), levonorgestrel (LNG), and etonogestrel (ENG). To identify the individual toxicity of each compound, juvenile female rainbow trout (*Oncorhynchus mykiss*) were intraperitoneally injected with either 0.001 nmol/kg, 0.0032 nmol/kg, 0.010 nmol/kg, 0.032 nmol/kg, 0.100 nmol/kg or 0.320 nmol/kg of EE2, LNG, ENG, or HPβCD n=10 fish per treatment. Blood, liver, gonads, and brains were sampled 7 days post injection and analyzed for changed in vitellogenin (VTG), endogenous steroid concentration, and alterations in gene expression. Next, juvenile female rainbow trout were injected with a mixture of HPβCD and either EE2, LNG, or ENG, and sampled 7 days after injection. Results are pending. Preliminary results from this study indicate that in the presence of HPβCD, the 96 h acute toxicity of EE2 to larval American flagfish (*Jordanella* floridae) was significantly reduced in a 1:1 molar ratio (EE2:HPβCD) ($P \leq 0.05$). Thus an interaction between HPβCD and EE2 appears to be able to be detected though a biological assay.

MP152 Toxicity of aqueous exposure to L-selenomethionine to early life-stages of fathead minnows

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Selenium (Se) is an important micronutrient involved in numerous metabolic functions in vertebrates. However, its uniquely narrow range between essentiality and toxicity has generated concern due to elevated environmental concentrations. While selenium naturally enters the environment at relatively low concentrations from geological sources, anthropogenic activities have significantly increased its release into aquatic ecosystems. Numerous studies have shown that oviparous vertebrates, fishes in particular, are highly susceptible to elevated dietary selenium concentrations during early development due to maternal transfer. During yolk reabsorption, it is hypothesized that larvae metabolize selenium-containing proteins to produce reactive compounds that interact with biomolecules such as lipids and proteins to induce oxidative stress. Exposure to seleno-L-methionine (SeMet), the predominant form of Se in the diet, during early life stages can induce teratogenic effects such as spinal deformities (lordosis, kyphosis, and scoliosis), edema, and fin and craniofacial malformations, as well as increase the incidence of mortality in developing fish larvae. Previous studies addressing the effects of selenium have largely consisted of dietary exposuresand embryo injections, with more recent work involving aqueous exposures. However, research investigating the effects of aqueous exposures on native fish species has received little attention. More importantly, research comparing the effects of embryo injections to aqueous exposures has yet to be conducted. Using *Pimephales promelas* as a model test organism, this study characterizes the effects of selenium (SeMet) on development of fathead minnow embryos. Fathead minnow embryos between 2-5 hours post fertilization (hpf) were aqueously exposed to SeMet (0, 3, 10, and 30 µg/L, six replicates/treatment, 25-30 larvae/replicate) for 6 days. In addition to mortality, characteristics such as spinal deformities (lordosis, kyphosis, and scoliosis), edema, and severity of fin and craniofacial malformations scored. Experiments are ongoing, with results of mortality and deformities allowing for comparison of sensitivity to developmental SeMet toxicity in native fathead minnow larvae.

MP153 Toxicity of triclosan and triclocarban to Daphnia magna: Effects of photolysis, dissolved organic matter, and mixtures

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Triclosan (TCS) and triclocarban (TCC) are two ubiquitous anti-microbial compounds commonly found together in surface waters. Both compounds have been shown to undergo photodegradation in surface waters, with TCS forming a variety of products including 2,8-dichlorodibenzodioxin, while the degradation products of TCC have not been clearly defined. Numerous studies have investigated the toxicity of the parent compounds, particularly TCS, but little information exists regarding the toxicities of the photolysis products for either compound or their mixtures. Lethality tests were conducted with *Daphnia magna* to determine 96-h LC_{50}s for both parent compounds in moderately hard EPA water, in the presence and absence of dissolved organic matter (DOM), and their photolytic products. Toxic unit (TU) equivalents were estimated from 96-h LC_{50} values for each compound and its photolysis products and 96-h mixture toxicity tests were conducted using 0.05, 0.1, 0.5, 1, and 2 TU concentrations and a control of USEPA moderately hard water. TCC toxicity was not affected by the presence of DOM but the toxicity of photolyzed products of TCC was enhanced by the presence of DOM. Photolysis decreased the toxicity of TCS and the presence of DOM did not alter the toxicity of TCS or its photolysis products. Of the six binary mixtures investigated, three showed synergistic toxic effects and three showed additive effects. The mixture of TCS+TCC (TU_{50} of 0.0246, 95% CL 0.0064- 0.6909) was about an order of magnitude more toxic than the mixtures of photolyzed TCC in DOM + TCC (TU_{50} of 0.4750, 95% CL 0.3401- 0.6641), and photolyzed TCC in DOM + TCS (TU_{50} of 0.5298, 95% CL 0.4063- 0.6909), all of which displayed synergistic toxicities. The mixtures of photolyzed TCC in DOM + photolyzed TCS in DOM (TU_{50} of 1.450, 95% CL 0.5344- 3.924), both TCS and TCC photolyzed simultaneously in the same solution (TU_{50} of 0.9870, 95% CL 0.3832- 2.541), and photolyzed TCC in DOM + photolyzed TCS in EPA (TU_{50} of 0.6790, 95% CL 0.2330- 1.981) were all statistically the same as a TU_{50} of 1, suggesting additive toxicity.

MP154 Transgenerational effects of early exposure to triclosan in zebrafish

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Worldwide, triclosan is among the most frequently identified component of pharmaceuticals and personal care products (PPCPs) within the aquatic environment. This antimicrobial agent used in a variety of PPCPs is a known endocrine disruptor, and readily bioaccumulates within aquatic organisms. Evaluating the potential population-level consequences of exposure to triclosan requires careful examination of the long-term effects following exposure during critical periods of development. Zebrafish were exposed to 0-40 mg TCS/L via static waterborne exposure with daily renewal during the period of metamorphosis and sex differentiation. Metamorphosis was delayed, likely due to disruption of thyroid hormones. Reproductive capacity was reduced, and unexposed offspring showed reductions in growth, survival, and metamorphosis. Taken together, our work suggests that chronic exposure to environmentally relevant concentrations of TCS poses a risk to wild fish populations, and warrants further study.

MP155 Reproductive and transgenerational effects of equilin in Japanese medaka and hepatic estrogen-responsive gene expression

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Limited data are currently available on the toxicological implications of equine estrogens in aquatic ecosystems. Our previous study has demonstrated transcriptional profiles of estrogen-responsive genes, such as *vitellogenins* (*Vtg1* and *Vtg2*), *choriogenins* (*ChgL* and *ChgH*) and *estrogen receptor* subtypes (*ERα*, *ERβ1*, and *ERβ2*), in the liver of male medaka (*Oryzias latipes*) exposed to six equine estrogens (1-300 ng/L) for 3 days. Results showed that the estrogenic potencies of the chemicals were in the order of equilin>17β -estradiol>equilenin>17β-dihydroequilin>17β-dihydroequilenin>17α-dihydroequilin>17α-dihydroequilenin, suggesting the higher lowest-observed effective concentration (LOEC) of 17β-estradiol (LOEC: 30 ng/L) than that of equilin (10 ng/L). In the present study, therefore, we investigated the reproductive and transgenerational effects of equilin in paired medaka exposed to 10, 100, and 1000 ng/L for 21 days. The short-term reproduction assay demonstrated that equilin (100 and/or 1000 ng/L) adversely affected the reproduction (fecundity and/or fertility) of adult medaka. In F_1 generation fertilized eggs, the hatching of embryos in the 100 and 1000 ng/L treatment groups also showed adverse effects. We further monitored the transcriptional profiles of estrogen-responsive genes in the liver of reproductive impaired male and female medaka. Our quantitative RT-PCR analyses revealed that exposure to 100 and/or 1000 ng/L of equilin upregulated the expression levels of hepatic *Vtg*, *Chg* and *ERα*/*β1* genes in males, whereas these treatments downregulated hepatic *ERα* gene expression in females. These results suggest that equilin causes the reproductive and transgenerational effects in medaka and sex-dependent responses of the hepatic estrogen-responsive genes.

MP156 Assessment of alternative lampricide treatments to protect lake sturgeon from non-target mortality

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The lampricide 3-trifluoromethyl-4-nitrophenol (TFM) is used to control sea lamprey populations in the Great Lakes. Typical treatments involve the application of TFM to larval lamprey nursery streams. TFM is normally applied at the 1.2-1.5 times, the 9-h $LC_{99.9}$, the minimum lethal concentration (MLC) needed to cause 99.9% mortality over 9h. Lake sturgeon are particularly sensitive to TFM during early development (juveniles < 15-20 cm), when their sensitivity is as great as that of sea lamprey. Since sturgeon have some capacity to detoxify TFM using phase II biotransformation (glucuronidation), we hypothesized that death resulted when their TFM detoxification capacity was overwhelmed. With the goal of protecting sturgeon from TFM toxicity during lampricide treatments, we investigated the effectiveness of an alternative TFM regimen, where lamprey and sturgeon were exposed to a lower concentration of TFM for a longer time. Consequently, ammocoetes and lake sturgeon were simultaneously treated with either the 9h or the 24h MLC of the lamprey, over 9 and 24 h, respectively. Lamprey mortality was 100% over the two treatments, while sturgeon mortality decreased from 65% during exposure to the 9h MLC, to < 5% during exposure to the 24h MLC. In addition, the lake sturgeon were able to detoxify TFM three times more efficiently during the long and low TFM regimen compared to the 9h MLC exposure. Consistent with earlier studies, sea lamprey exposed to the long and low TFM regimen showed only limited TFM detoxification capacity, but the sturgeon's capacity to convert TFM to TFM-glucuronide increased 3- to 5--fold between 9 and 24 h of exposure to the lower TFM concentrations. We conclude that the "long-and-low" TFM regimen may be an effective treatment alternative, that eliminates lake sturgeon mortality without compromising the effectiveness of the lampricide treatments.

Environmental or Analytical Chemistry – Part 1

MP157 Accurately Measuring Plant-based Activated Carbon in Sediment Samples also Containing Native Carbon

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The Lower Duwamish Waterway Group (LDWG) is conducting a pilot study on the effectiveness of enhanced natural recovery (ENR) amended with activated carbon (AC). Aspects of the study include the effectiveness of placement of the very fine grained AC in a thin sand or gravelly sand layer and determining any loss over time of the material once placed. The measurement of AC in the sediments is an important component of the project evaluation. Initial plans were to determine both Total Organic Carbon (TOC) and Black Carbon (BC) in the samples. The initial assumption was that the BC method would measure both background BC inputs from the system and the AC remaining in the placed layer. The form of AC used for the study was made from coconut husks and had a broad particle size range from granular to powdered activated carbon (1000-200 µm) to address various design needs. The AC made from coconut- and wood-derived carbon is very heat labile and its performance in the carbon analytical methods was unexpected. For example, BC methods based on pre-combustion at 375°F, such as those by Gustafsson et al. (1997), result in practically complete combustion of the plant-based AC in the pre-combustion step, with almost none surviving for measurement in the second combustions step of the method. The LDWG has been working with a number of laboratories on the modification of various carbon methods for quantification, including methods based on total volatile solids, TOC, and those that use pre-combustion or chemical treatment to remove specific fractions. The poster will outline the key differences in the methods, identify the fractions of carbon the methods were best at quantifying, and the effect of sample size and grinding, and will include final recommendations for working with AC from plant (wood and coconut)-derived materials.

MP158 Analysis of combustion byproducts on firefighter protection equipment and skin after exposure to smoke

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During a fire, firefighters are exposed to smoke that contains carcinogens and organic chemicals that can vary based on the burned material. In case of a house fire, flame retardants and polycyclic aromatic hydrocarbons (PAHs) and their combustion by-products can be found in smoke. While the firefighter gear is designed to protect from heat, smoke can make it through the gear onto the skin of firefighters. Organic contaminants can enter the body through inhalation when protective gear is not worn or through dermal exposure. High resolution MS is extremely advantageous when it comes to the analysis of the environmental contaminants in complex matrices, especially for identification of trace compounds. *In vitro* bioassays on the other hand can be employed for the evaluation of complex mixtures of known and unknown chemicals. Helmet, firefighter body wipes and urine samples prior to and after exposure to smoke were collected and extracted. GC/MS data were acquired using a novel 7250 accurate mass high resolution GC/Q-TOF system capable of low electron energy EI. The data were processed using MassHunter B.08.00 Qualitative Analysis software and Unknowns Analysis. Initial compound identification was performed by spectra comparison with NIST14 EI library and confirmed by Retention Index (RI) matching. An aliquot of the extract was also tested on two in vitro bioassays including an AhR activation assay and a p53 reporter gene activity assay. Preliminary data of urine samples showed elevated concentrations of hydroxylated polyaromatic hydrocarbons (PAHs), such as naphthols, fluorenols and phenanthrols after exposure to smoke. Multiple PAHs and polybrominated diphenyl ethers (PBDEs) were identified on the helmet and the skin wipes. Over 40 different brominated species (including PBDEs) and a dozen PAHs and their derivatives were identified in helmet wipes and skin wipes after exposure. In addition, the in vitro bioassay results show increased activity 2-10h after exposure compared to urine samples taken prior to exposure. Differences in monitoring results with respect to protective equipment are currently being evaluated.

MP159 Analytical Method for Routine Monitoring of Flame Retardant Tetrabromobisphenol-A-bis(dibromopropyl ether) in Various Matrices Using GC-MS

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Brominated flame retardants (BFRs) have been ubiquitous environmental contaminants for several decades now. Environmental monitoring agencies worldwide primarily use gas chromatography-mass spectrometry (GC-MS) for reliable, cost-effective routine BFR analysis. This technique utilizes chemical ionization in the negative mode to produce a unique signal for monitoring bromine-containing compounds. There are, however, limitations to GC-MS analysis of some BFRs, namely those of high molecular weight or thermal-instability. With recent regulations on the production and use of polybrominated diphenyl ethers (PBDEs) and other high production volume chemicals, alternative BFRs are being introduced as replacements with similar chemical structures, more bromine substitution, and higher molecular weights. For example, Tetrabromobisphenyl-A-bis(dibromopropyl ether) (TBBPA-BDBPE) is a derivative of one of the most heavily produced BFRs, tetrabromobisphenol-A. With a higher molecular weight and bromine substitution, TBBPA-BDBPE is susceptible to the limits of GC-MS analysis, i.e. thermal degradation. To overcome these challenges, the present work focuses on the optimization of the analytical parameters for the accurate and reliable determination TBBPA-BDBPE in various environmental matrices using GC-MS. Under optimized conditions, TBBPA-BDBPE was screened for in herring gull eggs, feces, regurgitates, soil, sediment, and earthworms collected in and around the Laurentian Great Lakes. Liquid chromatography-mass spectrometry was also explored to further validate the present GC-MS method and screen for potential degradation products of TBBPA-BDBPE.

MP160 Assessment of Human PAH Metabolic Profiling by Analyzing Multiple Urinary Deuterated Phenanthrene Metabolites in Exposed Humans

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Polycyclic aromatic hydrocarbons (PAH) such as benzo[*a*]pyrene (BaP) are believed to be causes of lung cancer in cigarette smokers and as a result of certain occupational exposures. PAH require metabolic activation for carcinogenesis. One pathway proceeds via bay region diol epoxides such as BPDE that react with DNA to produce pro-mutagenic adducts. Phenanthrene (Phe) is the simplest PAH with a bay region, but is not carcinogenic and therefore provides a good model for studying PAH metabolism in exposed humans. The diol epoxide pathway yields the urinary metabolites 1,2-dihydro-1,2-dihydroxy-1,2,3,4-tetrahydrophenanthrene (Phe-1,2-diol), *r*-1,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (PheT) and 1,2-phenanthrenequinone (Phe-1,2-quinone), whereas phenanthrols (HOPhe) signify detoxification. We are studying the metabolism of $[D_{10}]$ Phe in humans to assess pathways of PAH metabolism. The aim of this study was to develop a new method for the analysis of $[D_{10}]$Phe-1,2-diol and $[D_8]$Phe-1,2-quinone in human urine and to investigate metabolism by the diol epoxide pathway after oral administration of $[D_{10}]$Phe (1 µg dose and 10 µg dose). Administration of $[D_{10}]$Phe to humans is novel and unique, allowing us to acquire a more comprehensive understanding of the PAH metabolism pattern in humans, to monitor individual differences in PAH metabolism and possibly cancer individual susceptibility without interference from ubiquitous environmental exposure to Phe.

MP161 Chemical Monitoring and Toxicity Testing of Surface Waters in Georgia and South Carolina

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Seven locations across three rivers and two lakes in Georgia and South Carolina have been monitored with passive sampling devices (PSDs) for two and half years. Sampling periods were approximately four weeks in length. Earlier research showed differing, and significant responses from fish sampled at many of these sites using the yeast estrogen screening (YES), EROD, and GST bioassays. These results were proceeded by a long term chemical monitoring project using polar organic compound integrated sampler (POCIS) devices and low density polyethylene (LDPE) strips to analyze for estrogens, pharmaceuticals and personal care products (PPCPs), PAHs, and PCBs. POCIS extracts tended for estrogen analysis were derivatized with dansyl chloride and analyzed for estradiol (E2), estrone (E1), ethinyl estradiol (EE2), and BPA using UPLC-MS/MS. Underivatized POCIS extracts were analyzed for seven other PPCPs with the same instrument. Levels of PPCPs analyzed for in the Savannah River are estimated to be at ng/L to sub-ng/L concentrations, below physiologically relevant concentrations. PAH analysis was performed for the EPA Priority 16 using GC-EI/MS. Sites downstream of the Sangamo-Weston Superfund site were monitored for 128 PCB congeners using GC-ECD. Total PCB concentration ranged between 5-12 ng PCB_{TOT}/g PSD. The chemical profiles for each site varied between one another, as well as throughout the year. Overall concentrations were greater near more urbanized areas. For select samples from each monitoring site, Daphnia and zebrafish toxicity bioassays were used to assess the relationship between the unique chemical profiles and observed biological effects.

MP162 Determination of the absorption rate and partition coefficient of organochlorines and polyaromatic hydrocarbons in water using polydimethylsiloxane

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Many hydrophobic contaminants of environmental relevance such as organochlorines and polyaromatic hydrocarbons can have harmful health effects to humans and other living organisms due to their ability to disrupt endocrine functions at very low concentrations and their long persistence in the environment at trace levels. Hence, these compounds are not easily sampled by traditional sampling methods since these techniques require collecting large volumes of water samples at different times.To overcome this problem,polydimethylsiloxane (PDMS) can be used as a passive sampler due to its high affinity for a broad range of hydrophobic compounds. In this study,ten pre-cleaned polydimethylsiloxane pellets were used as passive samplers to determine the absorption rate and partition coefficient of 40 low solubility organochlorine compounds (OCs) and polyaromatic hydrocarbons (PAHs).The experiments were conducted in deionized water spiked with OCs and PAHs under controlled conditions during a three day period (collected every 3 h in a total of 58 h) with the goal of determining the partitioning behavior of these compounds between PDMS and water. In order to demonstrate the great potential of polydimethylsiloxane as absorbent of these two different categories of organic compounds and its use for measuring dissolved concentrations in the environment,the solid-liquid partition coefficient of each compound will be calculated and used to predict equilibrium concentrations in surface water.

MP163 Determining environmental concentrations of enantiomeric 2, 2', 3, 5', 6 polychlorinated biphenyls(PCB-95) for developmental neurotoxicity studies

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Of the various adverse health effects of polychlorinated biphenyls(PCBs), developmental neurotoxicity has emerged as a particularly vulnerable endpoint in PCB toxicity. But the underlying mechanisms have not been fully explored. First steps of far-reaching goal of studying the developmental neurotoxicity were to identify the current environmental concentrations of PCBs. Sediment samples analyzed in 2012 for total PCB concentrations with 59 surface sediment samples from Lake Huron were quantified for 39 PCB congeners in a previous study. The average concentration of total of 39 PCBs was 3.73 ng/g dry weights, and concentrations ranged from 0.05 ng/g to 90.9 ng/g dry weight. PCB-95 concenrations were detected at 0.13 ng/g. In Lake Hartwell (SC), concentrations in the top 10 cm ranged from 690 ng/g -3760 ng/g dry weight for the sum of 128 congeners measured in six cores collected in 2015. In particular, PCB-95 concentration was 0.14 ng/g and was detected mainly as a non-racemic PCB-95. This is strong evidence of stereoselective biotransformation. Studies on enantiomeric toxicity of PCBs are scarce and in particular very limited information available for PCB-95 which has been long considered as a potent neuro-toxicant. Therefore, the present study focuses on studying the enatiomeric effects of PCB-95 on early neurodevelopmet of an organism. Habituation assays are being conducted using *Danio rerio* (zebrafish) as a model organism. Previous behavioral studies have been conducted with Aroclor mixtures at concentrations ranging from 2-10 mg/L. We determined the concentrations of PCB-95 in Great Lake and Lake Hartwell samples to conduct realistic habituation assays with racemic PCB-95 and single enantiomers. Habituation assays conducted with racemic PCB-95 exposure to zebrafish dictated the marked differences in behaviors of the treated group compared to the controls. We expect different toxic potencies for PCB-95 enantiomers than those of the raemic mixture in neurodevelopment of zebrafish larvae. Outcomes from the preliminary testing shed lights on relvealing toxic effects at environmental concentrations and their underlying mechanisms of neurodevelopment deficits with prenatal exposure of non co-planar PCBs relavant to ecological consequences and human exposure.

MP164 Elucidating the characteristic of trace element and radioactive concentrations and ecological risk assessment in Ningyo-toge, Okayama

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One of the main uranium mine in Japan is Ningyo-toge. The radioactive levels in the sediment and the environmental water showed higher than the control sites, as residuals of uranium ore were measured. As a result of region comparison, Cr, Mn, Fe, Co, Ni, Cu, Zn, Se in sediments, As and Sr in environmental water were considered to be characteristic elements in Ningyo-toge. Therefore, implementing consecutive monitoring and more detailed analysis are needed to elucidate the influence of trace elements and radioactive in sediment and environmental water to organisms.

MP165 Elucidation of the trace element diffusion from a shooting range

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After it was established in 1983, the Tottori clay firing range was closed by the issue of environmental pollution with the lead bullet from 2008. For person of hunting upbringing that decreased year by year, from point of view that planned prevention of birds and beasts damage, re-maintenance was pushed forward. Administration was reopened in September, 2016. The pollution diffusive factor of the surface water and groundwater in the shooting range is used and recovered amount of lead bullet, impact ranges, the property of the soils, the rainfall situation and the flow situation of the groundwater. For these factors, the extent and moving of the trace element diffusion are very different. In this study, we aimed to elucidate the the trace element diffusion from a shooting range by (1) to measure trace element concentrations in river water and sediments around shooting range; (2) to analyze Pb isotope ration. As a result of comparing levels of surface water in the shooting range to river water, 9 elements (Co, Ga, As, Se, Se, Sb, Ba, Tl, Pb) were characteristic element of the shooting range. As a result of comparing river water levels of closing to opening, it was showed that Sb and Pb were released in environment after meintenace.

MP166 Fast routine analysis of polar ionic pesticides in water by suppressed ion chromatography and mass spectrometry

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Presence of polar pesticides and their metabolites in water and foods has become very hot topic in the past couple of years. The most famous representative of this group is a broad-spectrum systematic herbicide glyphosate and its metabolite AMPA. Because of the chemical properties especially high polarity, it is not possible to analyse these compounds with the conventional C18 column. Typically the laboratories use the methods that include derivatisation step or special chromatographic columns like the porous graphitic carbon (PGC) based Hypercarb. With both approaches varying method robustness and unreliable results are often reported by routine laboratories. We do present an Ion Chromatography Mass Spectrometry (IC-MS) method for direct analysis of five polar ionic pesticides (Fosetyl-Al, Glufosinate, AMPA, Clopyralid and Glyphosate) in water samples. IC is the preferred separation technique for polar ionic analytes such as anions, cations or ionic metabolites and thanks to the recent development in the hyphenation of IC and MS it enables its unproblematic usage. The method is very easy and fast with no need for a sample preparation. Only for very dirty surface water samples the filtration through the membrane filters is recommended. The method was validated in-house for three water matrices covering surface, drinking and bottled water. Analytical parameters as linearity, specificity, LOD, LOQ, precision and accuracy were evaluated using fortified blank water samples at three different levels. All tested parameters showed satisfactory results with LOQs well below required EU legislative limits.

MP167 High-Throughput Identification and Characterization of Microplastics Using Direct Analysis in Real Time (DART) Mass Spectrometry

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Polymers and plastics are produced in large quantities in modern society and widely used in a variety of industrial and consumer products. Microplastics are ubiquitously found in aquatic environments around the world. The lifetime of microplastics in aquatic environment can exceed that of most of the anthropogenic persistent organic pollutants. Due to concerns about risks to organisms from ingestion, plastic microbeads smaller than 5 mm in diameter have been listed as a toxic substance and subject to the regulations of the Canadian Environmental Protection Act (1999). Environmental risk assessments and potential regulations of microplastics require effective analytical methods that yield sufficient diagnostic information for plastic identification and characterization and also consideration of additives, leachable substances, and degradation products with respect to exposures. While current methods (microscopy, FTIR and Raman spectroscopy) have advantages in providing information for plastic identification, they do have some limitations including limited ability to identify and quantify additives and degradation products. The objective of this study is to develop direct analysis in real time (DART) mass spectrometry into a new and complementary tool for rapid and simultaneous analyses of polymers and additives in macro- and microplastics. The developed method was applied to characterize virgin plastic materials, plastic products, and microbeads in consumer products, and environmental samples. Based on the Kendrick mass defect, isotope patterns and MSMS spectra, plastic additives such as aliphatic amines and amide used as anti-adhesive and polymerization inhibition agents during plastic manufacturing were tentatively identified in the samples. Fragrance chemicals (e.g. Kephalis) widely used in cosmetic products were also found in some environmental microplastic samples. Due to the higher-throughput nature of the method, a greater number of microplastics particles may be analyzed for a much broad characterization of plastics. The spectra of polymers with known identifies were made into a fingerprint library, which were used to compare compositions with an environmental microplastic sample. Results indicate the method is promising and may help to further elucidate possible sources of plastics and perhaps eventually to the analysis of bulk environmental samples for plastics.

MP168 Monitoring source and drinking waters with a rapid and robust online LC/MS/MS method

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In 2015 the USEPA announced an age-dependent drinking water Health Advisory (dwHA) for the natural freshwater toxins, microcystins (MCs). For pre-school age children and adults, the MC dwHA values are 0.3 mg/L and 1.6 mg/L, respectively. Although the dwHA values are non-regulatory values, this announcement provides compelling health information that cannot be ignored. In parallel, EPA Method 544, a solid phase extraction/liquid chromatography tandem mass spectrometry (LC/MS/MS) method was released. Our goal was to create an online concentration LC/MS/MS method with 12 MCs that meets the EPA's quality assurance/quality control (QA/QC) criteria. MC concentrations were measured in samples from freshwater lakes and drinking water. Samples were prepared by three freeze/thaw cycles, centrifuging, and filtering through a 0.25 µm polycarbonate filter. Our LC/MS/MS platform included a Thermo Scientific EQuan MAX (online sample concentrator) and UltiMate 3000 (UHPLC) system and a TSQ Quantiva (MS/MS).

This method included 12 MCs with calibration curves from 0.5 – 500 ppt with R^2 values greater than 0.996. The MCs eluted between 2.2 – 5.2 minutes allowing for the analyses time to be 3] MC-RR, [Asp^3] MC- LR, MC-HilR, and MC-WR at concentrations above the low health reference level of 21 ng/L. Our data suggests that 1) by not including 12 MCs in Method 544, the true risk potential of exposure to MCs in drinking and recreational waters will be underestimated greatly, and 2) an untargeted microcystin occurrence study needs to be performed in the USA. Finally, our LC/MS/MS method reduces sample preparation, chemical usage, and instrument and preparation time while meeting EPA quality assurance criteria.

MP169 Multiple Hormone Analyses and Pregnancy Detection in 50 mg and 150 mg Skin Biopsy Samples of Beluga and Bowhead Whales: LC-MS/MS and ELISA Comparison

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Steroid hormone assessments in marine mammals provides important information for conservation and management, especially for threatened and endangered populations. Progesterone and testosterone ELISA analyses conducted in cetacean blubber have been validated in cetaceans to determine reproductive status of females and males, respectively. ELISA technologies are limited to detecting a single hormone per kit and often require an extract from as much as 75-150 mg of tissue. These assays also demonstrate high inter- and intra-variability within the same kit and between kits of different manufacturers. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) methodology offers the opportunity to detect multiple hormones from the same extract. LC-MS/MS analysis provides absolute hormone quantitation for multiple hormones without the drawback of cross-reactivity among steroid hormones inherent to the ELISA methodology. Previously we reported on the successful use of LC-MS/MS for the simultaneous detection of multiple steroids from 50 mg gray whale skin biopsies (including blubber). Here, we report on method optimization and quantitation of progesterone and testosterone via LC-MS/MS analysis in beluga and bowhead whales of known reproductive status using 50 mg and 150 mg skin biopsies. Tissue samples obtained from Inuit hunts were extracted for steroids, processed using gel permeation chromatography, and spiked with isotopically labelled internal standards for quantitation. Two ELISA kits (Arbor and Enzo), and the LC-MS/MS were successful at detecting pregnancy, but the LC-MS/MS method did so with a smaller (50 mg) sample. Testosterone was detectable at low levels using LCMS/MS and a 50-mg sample, with an increasing sensitivity as sample mass increased. Preliminary comparison among methodologies indicate that ELISA analyses (1) are accurate for pregnancy detection regardless of kit manufacturer or laboratory, but (2) may not provide sufficient sensitivity for establishing lower baseline hormone levels, and (3) may demonstrate higher intrinsic variability when compared to analytical methods. LC-MS/MS analyses are underway to investigate the relationship between sample mass and sensitivity, and to determine whether this technique may help standardize steroid hormone analysis in cetaceans by generating accurate and precise quantitation data that is comparable among laboratories.

MP170 Origin search of trace elements in Hino river system and primary toxic test of the river water using upstream fat minnow (Phoxinus oxycephalus jouyi)

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In the present study, We focused on trace element, nutrient concentrations, and aimed to Environmental impact Assessment and contamination status in the Hino River system water in Tottori. As a result, Total concentration of NO_2^- and NO_3^- were significantly higher than other stations in livestock wastewater (JS). Blast non-damage paddy field water (TN) and Blast damage paddy field water (TAN) are neighbors and only water sources differ. Total concentration of JS flows into TAN NO_2^- and NO_3^- were significantly higher than TN without JS flowing in. NH_4^+ concentrations were a median value of JS of 1.8mg/L, median value of the tributary Forest Development downstream (SKL) was 2.0mg/L. From this, surrounding SKL was suggested that NH_4^+concentration equivalent to JS flowed out. In Trace elements analysis in river water, 7 elements in JS and 5 elements in SKL were significantly higher than other stations. Especially, Manganese showed high levels, toxicity effect assessments was done. As a result, Althought the median of JS did not exceeded NOEC in rainbow trout embryos, the median SKL was 4.12 times higher. Moreover, Mn levels of SKL was 2.23 times higher than the LC_{50} in embryos of American frogs, the influence on the biota in the vicinity of SKL was concerned. There are few habitats in the area around the Forest Development site compared to other areas. Takahaya (Phoxinus oxycephalus jouyi) is also one of them. therefore, It is necessary to investigate whether river water around the forest development area has an effect on the habitat of this species. As a results, During this exposure period, Takahaya of SKU water exposure showed abnormal respiration and a decrease in activity in all individuals. Therefore, it is urgent to clarify the relationship between such abnormal behavior and Mn and Fe.

MP171 Synthesis of Various Biochars and their Applications in the Adsorptive Removal of Nitrogen from Water

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Excessive ammonium-nitrogen (NH_4^+-N) in water has created a serious concern worldwide. The nitrogen contamination occurs due to both point and non-point sources. High concentration of nitrogen causes various health problems such as methemoglobinemia, or blue baby syndrome. Therefore, it is imperative to remove nitrogen from water. This study focuses on the synthesis of biochars and their use as an adsorbent to remove NH_4^+-N from water. Three different biomasses namely; Wood shavings, Sugarcane Bagasse, and Goat droppings were pyrolyzed in a Non-stirred Parr Pressure Vessel at 350, 450 and 550 °C. The resultant biochars were characterized using Scanning Electron Microscopy (SEM), Brunauer-Emmet-Teller nitrogen physisorption method and Total Organic Carbon Analyzer to study morphology, specific surface area and total carbon content, respectively. Batch tests were conducted for 24 hours with initial NH_4^+-N concentrations of 5, 10, 50, and 100 mg/L for various adsorbents using a dose of 10 g/L to evaluate the effects of initial NH_4^+-N concentrations and adsorbents types on the adsorptive removal of nitrogen. Then, the amount of the adsorbents was increased to 40 g/L to identify the effects of adsorbent dose. Finally, adsorbents pyrolyzed at temperatures of 350, 450, and 550 °C were used to observe the effects of pyrolysis temperatures. At the end of each batch test, samples were collected and analyzed to determine the residual NH_4^+-N concentration using a HACH DR 2800 Spectrophotometer. Fourier Transform Infrared (FTIR) spectroscopy was also used to investigate the removal mechanism of NH_4^+-N onto the adsorbents. SEM micrographs reveal the chars as porous materials with large surface areas. Surface area and carbon content of the chars varied between 20 and 354 m^2/g and from 46 to 85%, respectively. The optimum initial NH_4^+-N concentration can be considered as 10 mg/L for adsorbents derived from sugarcane bagasse and goat droppings whereas 5 mg/L for adsorbent derived from wood. The percentage removal of NH_4^+-N increased with the increase of adsorbent dose from 10 mg/L to 40 mg/L for various adsorbents. The highest removal of NH_4^+-N was 78.5% with the adsorbent derived from goat dropping pyrolyzed at 450°C. FTIR results indicate insignificant changes in prominent functional groups confirming that physical adsorption has taken place. Bio-chars derived from wood, goat dropping and sugarcane bagasse can be promising adsorbents for NH_4^+-N removal from water.

MP172 The toxic mechanisms of silver ions and silver nanoparticles on airborne fungi

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Airborne fungi are ubiquitously distributed in our indoor and outdoor environment. Exposure to some fungal species has been reported to cause serious health problems, such as allergy, asthma, hypersensitivity.Recently, there has been increased attention toward the risk factors of the prevalence of fungi associated with respiratory diseases. Silver nanoparticles (AgNPs) have been used in numerous commercial products. Therefore, AgNPs are more likely to be exposed to humans and the environment. It is well known to have adverse effects on the survival and growth of various organisms, including bacteria, algae, cultured cells, and fungi, and animals. Many studies have focused on the inhibition effects of AgNPs and/or the ions released from AgNPs. However, the toxic mechanism for inhibition activity of AgNPs on airborne fungi is not fully understood. In this study, we have investigated the toxic mechanisms by AgNPs and the released silver ions on airborne fungi. We characterized the physic-chemical properties of the AgNPs by dynamic light scattering (DLS), Zeta-potential, and transmission electron microscope (TEM). We carried out the XTT assay to examine the viability of fungi.We also investigated the molecular mechanisms of Ag NPs on airborne fungi by real-time PCR.

MP173 Toxicity, Detection, and Formation Mechanism of Microcystin Oxidation By-products Resulting from Permanganate Treatment

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Over 160 congeners of the cyanotoxin microcystin have been identified in freshwaters across the world. These cyclic hepatotoxins, containing a conserved amino acid known as ADDA, are potent protein phosphatase inhibitors and suspected tumor promotors. In 2015, the USEPA announced a two stage age dependent Health Advisory (HA) for microcystins. The microcystins HA levels are 0.3 ug/L and 1.6 ug/L for children under the age of six, and adults, respectively. An accepted practice in the drinking water industry and governmental agencies is to use an ADDA specific ELISA test to determine total microcystin concentrations. Oxidative pretreatment of drinking source water with permanganate is widely used to reduce taste and odor compounds, color reduction, control zebra mussels, and to aid in the removal of contaminants containing unsaturated bonds and disinfection byproduct precursors. Permanganate's use for the oxidation of microcystins, has gained acceptance, but is also controversial in that it has been suspected that some of the partially oxidized microcystin by-products produce positive results with the ADDA-ELISA test. Our goal was to identify, isolate, and characterize the microcystin permanganate by-product(s) that interfere with the ADDA-ELISA. The kinetics of microcystin-LA destruction by permanganate ion and the formation of oxidation products were determined by LC/MS/MS. We have confirmed the mechanism by which microcystin-LA reacts with permanganate, and have identified the major oxidation products by LC-qTOF. These oxidation products were isolated and purified. Apparent toxicity of these oxidative products was assessed by the protein phosphatase inhibition assay. The results of the protein phosphatase assay were compared to the response with the ADDA-ELISA. The major observation is that the oxidation products do not show PP inhibition but still induce a positive response from the ADDA-ELISA. This can result in false positive ELISAs and lead to unnecessary drinking water advisories.

MP174 Detecting sulfamethoxazole and carbamazepine in a surficial sand-and-gravel aquifer: Is ELISA a reliable screening tool?

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Human activities have been recognized as introducing micropollutants into the aquatic environment. Micropollutants are commonly detected using mass spectrometry-based analytical methods, which are expensive and time consuming. Enzyme-linked immunosorbent assay (ELISA) methods are an inexpensive analytical alternative that provide semi-quantitative results in a quicker timeframe. In groundwater collected from nine monitoring wells located in surficial sand-and-gravel aquifers near land application sites, the use of ELISA was investigated for two commonly detected micropollutants, sulfamethoxazole (SMX) and carbamazepine (CBZ). The ELISA results were compared to two mass-spectrometry-based methods: (1) direct aqueous injection-high performance liquid chromatography/tandem mass spectrometry (HPLC) and (2) online solid-phase extraction with liquid chromatography/electrospray ionization-mass spectrometry (SPE LC). The Paired Prentice-Wilcoxon test was used to analyze differences in SMX and CBZ observations between ELISA and both HPLC and SPE LC. Estimates of bias and limits of agreement between paired observations were also calculated. Sulfamethoxazole determinations by ELISA yielded similar results to HPLC, but were 13% greater than SPE LC. Carbamazepine determinations by ELISA yielded results that were 29 and 9% greater than HPLC and SPE LC, respectively. The ELISA determinations were in agreement with HPLC for 88 and 80% of samples for SMX and CBZ, respectively; and 76 and 80% with SPE LC for SMX and CBZ, respectively. Results indicate that ELISA has the potential to be a reliable and cost effective alternative to more commonly used mass spectrometry-based analytical methods.

MP175 Evaluation of the level of organochlorine pesticides contamination of soils around Olusosun dumpsite, Ojota Lagos

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Olusosun dumpsite is the largest in Africa and one of the largest in the world. The site receives up to 10,000 tons of mixed wastes each day. Organochlorine pesticides (OCs) present in the dumpsite could be accumulated in biological tissues and the subsequent magnification of concentrations in organisms, progressing through to the food chain may pose potential danger to the soil organisms. Emission into surrounding water bodies and their possible translocation into edible parts of crops is of great concern. OCs constitutes eight of the twelve persistent, bioaccumulative and toxic micropollutants (PBTs) identified by UNEP as the "dirty dozen". This means that the negative health and environmental impacts of OCs from Olusosun landfill site, now surrounded by commercial and residential areas, could be significantly high. Soil samples were collected from the Olusosun dumpsite Ojota, and analysed for organochlorine pesticide residues. Extraction of the soil samples was carried out using the EPA 3570 and analyzed for eighteen OCs using gas chromatography-mass spectrophotometry (GC-MS). The OCs were found to be unevenly distributed between the surface, middle and lower soils. In the surface soil cis-permethrin was highest (176.57ppm). In the middle soil, endrin was highest (265.75ppm). In the lower soil, endosulfanII was highest (114.61ppm). The physicochemical properties of the soil samples were also tested. A pattern was established which showed the total OCs highest (1749.54 ppm) in the surface soil, followed by the middle soil (487.50ppm), then lowest (297.73ppm) in the lower soil. The level of OC contamination of soils around Olusosun dumpsite is hereby presented.

Integrated Environmental Assessment and Management

MP176 Approaching Risk Assessment by remote perception to environmental vigilance

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As consequence of urbanization and industrialization, the upper basin of Atoyac River in Mexico has been presenting an accelerated environmental degradation, particularly by pollutant emissions and wastewater discharges from three industrial corridors. The health problems in small populations such as Texmelucan, Villa Alta, San Baltazar and Sta. María have been linked with pollution around of Atoyac river. The most frequency diseases reported in the area are asthma, thrombocytopenic purpura and leukemia in children less to 15 years old. In addition, inhabitants showed high blood genotoxic damage frequency, even above to Citizens of Mexico City in 2006. According to air monitoring program (ProAire), since 2006 air quality had showed a reduction of atmospheric pollutants (PM10, PM2.5, SO2, CO) in Puebla. However, the NO2 has been up to maximum limits of health protection with 79,164 ton per year. The ProAire considers the metropolitan zone of Puebla city only aside communities where industrial activity is intense but there is not installed any monitoring station. Our proposal is to apply new approximation to air monitoring in small cities as well as to generate a risk map. In this work, we delimit the zones exposure of contaminants using the molecular density in air column of NO2 between 2005 and 2012. We downloaded data form the Ozone Monitoring Instrument (OMI) on board of Aura Satellite and published in the Goddard Earth Sciences Data and Information Services Center (GESDISC) of NASA. To generate maps of pollutant dispersion, we used MIRADOR 1.42 tool and localized the sources of pollutions by molecular density. We contrasted the direction and speed win to observe dispersion patterns and established the ranges of minimum and maximum exposure. The information taken from the NO2 was supplemented with toxicity data by contaminant and marginalization to generate the risk map, to identify places where it is plausible to find environmental problems, with this information we could observe the possible links between exposure to pollutants and biological effects.

MP177 Assessing the sensitivity of ecosystem functional and structural responses to chemical stressors: A meta-analysis

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Ecosystem structure and function are commonly used to assess the effects of stressors at the ecosystem scale. It is understood that these endpoints have different sensitivity to stress, and the assumption is that structural measures respond at lower stress levels than functional measures. These assumptions have influenced hypotheses in stress ecology as well as monitoring and management approaches, but the extent to which structure–function sensitivity has been empirically tested is not clear. We conducted a meta-analysis with the objective to assess the scientific evidence on responses of ecosystem function and structure to chemical stressors. Specifically, we aimed to examine 1) how frequently are functional and structural responses to stress empirically measured at the ecosystem-scale, 2) what are common metrics of ecosystem function and structure, and 3) if the sensitivity of these endpoints is related. We identified over 3,600 publications that included mentions of ecosystem structure and function in response to stressors such as chemical pollution and landuse change. Our selection criteria excluded manuscripts with no new empirical data, no measurable stressors, and studies that did not measure both structural and functional variables, which resulted in 370 publications included in this analysis. Metrics were considered functional if they represented rates of biological processes, and structural when they characterized a static measure of the ecosystem. Our initial analysis showed the most commonly used structural metric was community composition, whereas decomposition, respiration and primary production were the most common functional responses, respectively. Interestingly, nutrient transformation processes (e.g., nitrification) were seldom assessed within the analyzed studies. Assessment of metal-induced stress on function and structure accounted for 12% of our reference library, of which copper was directly measured in 10 studies. In order to conduct a subset meta-analysis on copper effects, we extracted structural and functional measures in response to copper stress and calculated log response ratios. Notably, we did not detect any significant differences in sensitivity in structural and functional log response ratios relative to copper concentration. Our goal is to continue this analysis across stressor types and expose any sensitivity patterns, or lack thereof, in ecosystem functional and structural responses to stressors.

MP178 Comparative Risk Assessment (CRA) of Response Options for a Deepwater Oil Release

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During the Deepwater Horizon accident, subsea dispersant injection (SSDI) was used as a countermeasure for this uncontrolled deep water oil release. Since 2010, several studies have indicated that SSDI can be more effective at reducing floating oil slicks than dispersant treatment at the water surface. This paper summarizes a year-long comparative risk assessment (CRA) project, funded by industry and involving frequent feedback from the US regulatory community. A 3-dimensional oil spill transport and fate modeling was combined with data on ecological effects to evaluate comparative exposures to and recovery of components of the ecosystem. The model considered various combinations of SSDI application at the source, mechanical recovery, in-situ burning, and dispersant application on the water surface. The objective was to provide decision makers with objective, science-based, and transparent information to enable technically-sound choices regarding appropriate strategies for mitigating impacts from oil and gas released during a deepwater blowout. Given this context, an engagement process was established with stakeholder representatives, decision makers, and others, throughout the project. The CRA evaluated potential trade-offs from increased offshore and nearshore water column exposures of biota to oil components in the water column resulting from dispersant use compared to increased exposures to oil on the water surface and on the shoreline without it. Oil spill trajectory model simulations were used to quantify the amount of shoreline, the area of the water surface, and the volume of the water column that exceeded defined oil exposure thresholds. Results demonstrate the value of including SSDI among available oil spill response technologies. SSDI can reduce the volume of oil that reaches the surface; reduce the volume of oil reaching nearshore ecological receptors; reduce exposure to volatile organic compounds (VOCs) for emergency response workers and biota; and reduce exposure of slow-to-recover wildlife to surfaced oil and VOCs.

MP179 Cross-phenomenon Between Dose-response Curve and Concentration Addition Curve of Sulfachloropyridazine-Erythromycin Mixture on Escherichia coli

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Human beings have been exposed to an environment that contains complicated and toxic chemical mixtures, which seriously threatens human health and global economic development. To determine the potential for harm and propose safety measures of chemical mixtures, concentration addition (CA) model has been widely used to predict and judge their joint toxic actions (synergism, antagonism and addition) by comparing actual dose-response curve (DRC) with the curves of CA curve. Among these studies, some researchers found that DRCs of chemical mixture crossed CA curves, named as "cross-phenomenon" that was characterized by a heterogeneous pattern of joint toxic action. However, mechanistic investigation on this phenomenon is extremely limited. In this study, cross-phenomenon between CRC and CA curve of Sulfachloropyridazine (SCP) and erythromycin (ERY) on Escherichia coli (E. coli) was observed at 12 h, expressed as antagonism in the low-concentration range, addition in the medium-concentration range, and synergism in the high-concentration range. Furthermore, this cross-phenomenon varied with increasing exposure time within 12-24 h: antagonistic effect of the mixture at a low concentration switched to synergistic effect, and the joint toxic action of the mixture at the high concentration changed from synergism to antagonism. Mechanistic explanation for this time-dependent cross-phenomenon was as follows: SCP and ERY as antibiotics could form a double block to inhibit the bacterial growth, exhibiting a synergistic effect; meanwhile SCP induced obvious hormesis on E. coli in the low-concentration range by stimulating the sdiA mRNA expression that could promote RNA polymerase binding to gene promoters to enhance the growth of E. coli, which increased the expression of the efflux pump (AcrAB-TolC) to discharge ERY, exhibiting an antagonistic effect. Because both the concentration and the extent of stimulation of SCP on the sdiA mRNA were increased with exposure time, this cross-phenomenon was observed to be a time-dependent process. This work explains the dose-dependent and time-dependent cross-phenomenon and provides evidence regarding the interaction between hormesis and cross-phenomenon.

MP180 Ecological and Toxicological Parameters for Arctic Spill Impact Mitigation Assessment (SIMA)

H. Robinson, M.J. Bock, R.J. Wenning, Ramboll Environ

With increased interest in polar shipping routes and oil and gas development in the pan-Arctic region, there is also increased occasion to develop scientifically sound and plausible comparative risk assessments (CRA) to support oil spill response (OSR) planning and preparedness. A sufficiently robust scientific and ecological information base is emerging that supports meaningful spill impact mitigation assessment (SIMA), an adaptation of net environmental benefits analysis (NEBA) for oil spill response in the Artic. SIMA and similar CRA approaches provide responders with a systematic method for determining ecological resources at risk and their sensitivity to oil exposures, comparing and contrasting the relative environmental consequences of different response alternatives, and identifying strategies during planning that minimize impacts and maximize the potential for a more rapid environmental recovery in the event of an accident. We compiled and summarized 3,500+ literature references describing Arctic ecosystems and the fate and effects of oil and treated oil on aquatic habitats and biota. The literature provides toxicity data, field observations, and life history characteristics of resources at risk for different spill conditions and oil types relevant to Arctic environments. This information was examined to identify a set of environmental parameters and a range of plausible assumptions for use in Arctic SIMA. These parameters were used to support screening-level SIMA evaluations of the consequences of oil spill events and different response options. This work is captured in an open-access internet portal developed to facilitate access to the scientific literature and key considerations supporting SIMA in the Arctic environment.

MP181 Effect of seasonal soil temperature on the biological degradation of estrone and triclosan mixture by soil microbial community

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Pharmaceutical and personal care products (PPCPs) are often not completely removed by the process of waste treatment plant. PPCPs associated with land farming of municipal wastewater effluent may potentially persist as mixtures in the soil and alter soil microbial community processes. The microbial community in the soil environment is responsible for many activities relating to soil fertility and decontamination of the soil environment through natural attenuation or bioremediation, when it is exposed to anthropogenic contamination. The soil microbial community composition and physiology and the influence they have in decomposition and nutrient availability is affected by soil temperature. Soil with a long history of exposure to these compounds through wastewater effluent was examined at different seasons (summer and winter) to evaluate the effect of seasonality on the biological degradation of estrone and triclosan. The soil samples were spiked with estrone, triclosan, and a 1:1 mixture of estrone: triclosan and incubated for 90 days at room temperature. Control samples consisting of unspiked soil were included in the analysis to account for contaminate effects vs. seasonal effects. The community level physiological profile was examined using BIOLOG® EcoPlates™ for the ability of their microflora to utilize ecologically relevant carbon sources. Substrate activity (SA) and substrate richness (SR) was determined from this analysis. Microbial degradation rates were compared over the 90 days incubation period using high performance liquid chromatography (HPLC) and half-lives of each compound from the different soil conditions determined. Soil microbial community composition is affected by seasonal temperature changes, which thus affects the rate of degradation of estrone, triclosan and their binary mixture at different soil conditions.

MP182 Effectiveness of a Constructed Wetland for the Tertiary Treatment of Nutrients in Municipal Wastewater During the First Year of Establishment

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Incomplete treatment of wastewater prior to discharge can compromise the water quality of downstream communities and impair receiving aquatic systems. Moreover, efforts to recycle treated wastewater effluent back into the municipal water source are often hampered by negative public perceptions of the effectiveness of traditional treatment, especially in small rural communities. Constructed surface flow wetlands offer a low-maintenance and affordable addition to traditional wastewater treatment for rural and undeveloped areas and have the potential to treat municipal wastewater beyond EPA discharge standards. This research examined bacteria and nutrient levels in a constructed wetland used in the tertiary treatment, or "polishing" of lagoon-treated municipal wastewater in the small rural setting of Sewanee, Tennessee. Effluent samples were collected from the final discharge of the current lagoon system and in four locations representing different treatment stages in a new surface flow constructed wetland. Total coliforms, *E. coli*, nitrogen, and phosphorus concentrations were quantified monthly between following wetland commission between June 2016 and July 2017. Throughout the sampling period, and across four seasons, total nitrogen and ammonia, as well as *E. coli* and coliform bacteria, were significantly reduced from the lagoon outflow to the wetland outflow. Nitrate concentrations remained very low throughout the system. A trend was observed for total and reactive phosphorus with levels decreasing as effluent passed through the wetland. Reductions in nitrogen and bacteria concentrations continued under reduced flow throughout the winter months when the wetland plants were dormant. These data demonstrate that during the first year of establishment, wetland processes represent an effective tertiary treatment to reduce nutrients and bacteria that would otherwise be discharged into the environment from the current lagoon system. The monitoring results will be shared with the Sewanee community in order to reframe the debate about the effectiveness of wastewater treatment and the potential for more sustainable water management through wastewater recycling.

MP183 Environmental Statistical Software - Pros and Cons

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Deriving summary statistics such as the mean, standard deviation, exposure point concentrations, and an upper confidence limit on the mean is straightforward when data are consistent and follow a specific distribution. However, environmental data are often complicated and messy. In risk assessment, 95% Upper Confidence Level on the Mean (UCLMs) are predominantly used as exposure point concentrations (EPCs). The U.S. Environmental Protection Agency (USEPA) developed ProUCL, a statistical software package with statistical methods and graphical tools that can be used to address many environmental sampling and statistical issues. ProUCL recommends a 95% UCLM based on data distribution, dataset size, skewness, and percentage of non-detect observations. In some cases, ProUCL may suggest more than one UCLM estimate. In these cases, a risk assessor evaluates the dataset and selects the most appropriate 95% UCLM. While the program is user-friendly and used nationally and internationally, using ProUCL has some drawbacks, specifically when working with large datasets with an inadequate number of unique values (*i.e.*, mostly non-detects or 1 to 2 detects) and small datasets (N < 10). Secondly, although ProUCL utilizes various parametric and non-parametric methods for estimating a 95% UCLM, the software tends to incorporate parametric method assumptions based on specific distributions (*i.e.*, normal, gamma, log-normal) as well as specific non-parametric methods based on unknown distribution. Lastly, though ProUCL estimates values using all of the available methods, there are instances in which no value may be calculated for a particular method. The aim of this poster is to explore the pros and cons of ProUCL compared with other software/methods for calculating UCLMs.

MP184 Factoring Pesticides of Emerging Concern in the TMDL Process

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Most of the arthropod invertebrates used for aquatic toxicity testing are sensitive to pesticides, which account for much of the toxicity in California state and regional monitoring programs. Toxicity and chemical analyses have led to 303(d) listings and pesticide-related total maximum daily loads (TMDLs) in many California water bodies. The succession of new pesticides has historically outpaced monitoring and regulation. TMDLs for the organophosphate chlorpyrifos were developed in four central coast watersheds between 2011 and 2014, but monitoring data from several programs demonstrated that pesticide usage in these watersheds has changed to pyrethroids, and more recently, to neonicotinoids. California is taking steps to get ahead of the pesticycle. The California Department of Pesticide Regulation (DPR) has developed a pesticide prioritization model that incorporates pesticide use as well as chemical properties, monitoring results, and application information at different spatial and temporal scales. Coordinated and cooperative monitoring has also been implemented to speed the transfer of information among state and federal agencies. In collaboration with DPR, the State Water Board is conducting water column toxicity tests at DPR surface water monitoring locations to determine if water toxicity is caused by current-use pesticides, such as neonicotinoids, or other pesticides of emerging concern. As the patterns of pesticide use have changed, the test species used in monitoring must evolve to assess risk to aquatic life because of differing sensitivities among standard test species. An additional test species was added to the State Water Board's Stream Pollution Trends Program (SPoT) to address potential sediment toxicity associated with fipronil. These activities are also informing the design of a statewide urban monitoring framework for pesticides and toxicity as part of the State Water Board's Strategy to Optimize Resource Management of Storm Water (STORMS). Successful or meaningful reduction of pesticides in urban and agricultural environments can only be confirmed with up-to-date monitoring tools that include biological measurements and analytical chemistry. This includes the development of next generation bioanalytical tools that have cell lines with neurotoxin receptors. This presentation will discuss examples of changing pesticides, and suggest preemptive strategies for avoiding negative outcomes.

MP185 Impacts of Climate and Environment on the Risk Assessment of Engineered Nanomaterials

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The use of engineered nanomaterials (ENMs) has rapidly expanded in recent decades. Predicting the impacts of these materials on human health and the environment is inhibited by limited data for their production, transport, fate, and toxicological affects. In this presentation, a risk assessment was performed for 6 major cities within Europe and the United States with the best available data sets and the Chemical Life-Cycle Collaborative (CLiCC) tool, a rapid spatially-explicit life cycle assessment model with little input information required. Specifically, the effects of environmental variability between the following cities was explored: Rome, Italy; London, United Kingdom; Zurich, Switzerland; and Los Angeles, New York, and Des Moines within the United States. These regions were also selected to compare anticipated effects of ENMs in cities within coastal Mediterranean climates (Rome and Los Angeles), cities in coastal environments with cold winters (London and New York City) and landlocked cities with a large freshwater lake sink (Zurich and Des Moines). In addition to the impacts of climate variability, the influence of regional soil, land use, population, waste treatment, and waste disposal methods were investigated.

MP186 Integrated Framework of Life Cycle Assessment and Biogeochemical Modeling (DNDC) to support Contaminated Site Management in Alberta

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Abandonment and reclamation of well sites and related infrastructure in Alberta needs to occur in a timely fashion to minimize environmental and fiscal liabilities. Abandonment rates of oil and gas well sites continue to outpace reclamation, contributing to ongoing environmental impacts. This is mostly because the extent of required remediation due to the complex pollutant pathways at the time of closure of the well is not correctly predicted and included in the life cycle of the asset, as such, operators are faced with huge costs of remediation and thus, walk away. This study seeks to improve the predictability of pollutant pathways and impacts on well sites by combining Life cycle assessment (LCA) and Denitrification Decomposition (DNDC) model in an integrated framework. Since LCA is difficult to evaluate soil-water-plant interaction, this study combines LCA with DNDC, a biogeochemical model which is essential in coupling land-water-atmosphere interactions in order to study dynamic interactions among nutrients, water, soil, vegetation and climate both above and below ground, thus, predicting the magnitude of contaminants for different pathways. By using an integrated model, greater levels of understanding can be reached on how to analyze and evaluate the interaction of complex processes. The framework is validated using an existing data. The model performed considerably better in simulating lower rates of contaminant degradation (< 0.1) compared to rates above 0.1. Different scenarios are modeled to optimize the model for assessments of reclamation practice. This will improve the understanding of reclamation, help identify key factors and processes, and predict much better the extent of future reclamation that may be required by operators of oil and gas wells, thus, reducing the number of abandoned wells in Alberta. This framework will also help us design and implement more effective sampling and monitoring systems, and propose or adjust measures for adaptive management of reclamation.

MP187 Life cycle impact of nanosilver polymers-food storage containers as a case study

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Some of the characteristic properties of silver metal include reflectivity, malleability, electricalconductivity, high ductility, and antimicrobial efficacy; due to these attributes, a world-wide estimated of887 million ounces of silver were mined during 2015. A percentage of this mined silver mineral is used tomanufacture nanosilver-enabled products, such as food storage containers. The antimicrobial properties ofnanosilver particles (AgNP) embedded in these products help to prolong the freshness of the stored food.This characteristic property of AgNP has stimulated an increase in AgNP-enabled products in the lastseveral years; with more than 25% of consumer nanoenabled products having nAg. Studies have foundthat AgNP migrates from the polymers comprising the food storage containers into the stored food. Theinitial AgNP concentration in these products ranges between 3.3 µg/g to 100 µg/g (mass of AgNP permass of container) with an average nanoparticle diameter of 10 nm - 300 nm. The migrated AgNPconcentration varies in function of the experimental conditions, nevertheless values as 42.5 ng of AgNPper cm2 of polymer have been observed. This presents the potential for ingestion of AgNP, through theconsumption of food stored in these containers. This work investigates the trade-off of maintaining the freshness and consume ability of stored food withthe environmental and human health impacts of utilizing AgNP-enabled food storage containers. Lifecycle assessment (LCA) is utilized in a cradle to grave analysis (including raw materials acquisition,manufacturing, use, and end of life) to compare the environmental and human health impacts of the AgNPenabled containers with their conventional counterparts. Estimates as to the potential reduction in foodwastage due to reduced spoilage are also generated and presented. The results of this work can help on thedeveloping of federal legislation with a more accurate scope, which can allow the completely sale ofregulated nanosilver-enabled food storage containers or the prohibition of such.

MP188 Looking beyond default inputs to risk-based remedial options - maximizing cost effective solutions

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Remedial options were evaluated for an ADEM industrial site in Birmingham, Alabama, using the ARBCA V2.2 risk modeling software. The risk assessment results were used to inform the risk manager on which exposure areas required remediation based on unacceptable levels of risk for the potentially complete exposure pathways. Polynuclear aromatic hydrocarbons (PAHs), including benzo(a)pyrene, were historically risk drivers for the site. The default ARBCA input parameters for toxicological criteria and exposure assumptions were used to model the potential risks to facility workers, construction workers, and trespassers except for exposure parameters related to exposure frequency and duration. Based on the potential unacceptable risks to the identified receptors, a baseline remedial approach that included selected soil removal and construction of impervious caps was recommended. It was noted that benzo(a)pyrene was a significant risk driver of the unacceptable risks. A review of the toxicological criteria in the ARBCA model for benzo(a) pyrene noted that they were based on toxicological values available at the time of software publication in 2015. The oral slope factor for benzo(a) pyrene in the model was based on an EPA Integrated Risk Information System (IRIS) Toxicological Review for Benzo(a)pryene in 2010 of 7.3 $(mg/kg\text{-}day)^{-1}$). However, in January 2017, the EPA IRIS issued a Final Toxicological Review with the oral slope factor of 1.0 $(mg/kg\text{-}day)^{-1}$. The ARBCA risk model was re-run using the current oral slope factor, and the previously unacceptable risks to the identified receptors were reduced in selected exposure areas to acceptable risks. Therefore, the revised remedial approach resulted in a reduced remedial effort in both selected soil removal and construction of caps. Further, significant uncertainty is inherent in the dermal absorption fraction (ABSd) exposure parameter for PAHs, and benzo(a)pyrene specifically, because of limited ABSd data due to different soil types, loading rates, and chemical concentrations in the experimental data. A sensitivity analysis was conducted using the EPA recommended 13 percent for ABSd and a ABSd of 6 percent used by other regulatory agencies. The results indicated ABSd had a moderate to high degree of sensitivity to risks from dermal exposure, and provide a range of remedial options the risk manager can use in the overall remedial strategy for the site.

MP189 Persistent Organic Pollutants and Risk Assessment

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The main task of risk assessment is obtaining and generalizing information on impacts towards the environment and human health with the ultimate aim to eliminate, decrease, regulate and monitor risks at different levels of exposure. Risk analysis includes such elements as: assessment of risks posed by such hazardous chemicals as persistent organic pollutants (POPs), in particular – Dioxins and Furans, to human health; risk management and risk notification. Appropriately, risk assessment requires scientific consideration of impacts and/or factors, as well as setting forth permissible impact levels. Estimation of danger is done based on values of individual and population risks, e.g. carcinogenic risks. This latter requires availability data on concentrations of hazardous substances and especially POPs. POPs as chemical compounds of high stability, ability for intra-media penetration and spatial distribution pose great danger for both human health and the environment. This group includes polychlorinated dibenzodioxines and dibenzofurans, polychlorinated biphenyls, organochlorine pesticides. Upon evaluation of chemical exposure risks to human health the assessment of “exposure – response” “dose – response”, “concentration – response” becomes an important determinant and allows to establish “cause – effect” determination of adverse effects development under the influence of a substance, as well as identification of the lowest effect doses. Upon assessment of the ratio between dose and reaction of an organism it is considered that: the level of response depends on the dose of a chemical; the higher the dose, the higher is the percent of population responding to the chemical exposure; the higher the dose, the severer is the response of a human organism; non-carcinogenic effect of chemical exposure is manifested only upon achievement of maximum (threshold) dose levels; threshold doses for carcinogenic effects cannot be estimated in theory

MP190 Probability Assessments of Mercury and PCBs in Great Lakes Fish

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In 2010, the U.S. Environmental Protection Agency (EPA) introduced a new approach for assessing chemicals in Great Lakes fish using a probability-based survey. This survey generated the first statistically representative data on the occurrence of mercury and PCBs in fillet tissue. EPA repeated the survey in 2015 to evaluate temporal trends. Fish were collected from 157 and 152 randomly selected U.S. nearshore sites throughout the Great Lakes in 2010 and 2015, respectively. Fillet samples were analyzed for total mercury and all 209 PCB congeners. All samples contained detectable levels of mercury and PCBs, with maximum mercury concentrations of 956 and 557 ng/g in the respective studies. Total PCB maximums were 2,380 ng/g in 2010 and 1,168 ng/g in 2015. Probability distributions from 2010 showed that fillets in 11% of the sampled population (representing 11,091 km^2 of nearshore area) exceeded EPA’s 300 ng/g methylmercury fish tissue criterion, and 13% of the sampled population (17,288 km^2) exceeded the criterion in 2015. Using Great Lakes Sport Fish Advisory Task Force (SFATF) consumption limits, 60% and 59% exceeded the 110 ng/g (one meal/week) threshold in 2010 and 2015, respectively. Most of the sampled population had total

PCB concentrations above one meal/week limits, including 99% above EPA's 12 ng/g human health (HH) cancer risk-based limit in 2010 and 79% in 2015, 88% exceeding EPA's 47 ng/g noncancer HH limit in 2010 and 53% in 2015, and 82% exceeding the SFATF 60 ng/g threshold in 2010 and 47% in 2015.

MP191 Research Strategies to Engage Communities in the Analysis of Lead Contamination of Water Supplies in the Mississippi Delta

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This project includes community-based participatory research and an assessment of residential drinking water supplies and water supply infrastructure in the Mississippi Delta. Our goal is to assess multiple social science approaches to engage stakeholders and influence policy on the current state of lead contamination in drinking water in Mississippi. The 2016-2017 cohort of students enrolled in the Tri-County Workforce Alliance and their parents served as our initial community partners. The participants came from four counties (primarily Coahoma) and 14 municipalities and all reported being on public water systems (e.g. not wells). Participants collected their home drinking water (first catch of the day from kitchen sink, cold water) and samples were analyzed for pH and lead concentrations. Sixty-eight of the 87 distributed bottles (78%) were returned. The pH of the drinking water samples ranged from 7.04-8.23. Notably, lower pH is associated with higher potential to leach lead. Of the samples tested from the Delta cohort, only 20 of the samples had lead concentrations above the detection limit, with the highest concentration being 3.45 ppb. All concentrations were well below the EPA 15 ppb action level. Letters were sent to each participant notifying them of their water results. Demographic data is being analyzed for risk factors associated with lead detects, and additional community cohorts are being engaged. For example in the cohort, 85% of the residences were houses (vs. apartments or mobile homes) and 47% of the respondents who estimated the age of their home indicated that it was built before 1985. Ultimately, this project has the potential to help safeguard public health because survey and sampling results will help assess the risks of lead contamination in the Mississippi Delta, assist with the identification of lead service lines and lead plumbing within the distribution systems, and design and guide scalable research and outreach efforts to minimize lead exposure through use of filters and/or behavioral changes.

MP192 Source identification and health risk assessment of PM2.5-bound PAHs in urban center of Beijing, China

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Polycyclic aromatic hydrocarbons (PAHs) are one group of crucial toxic components bound to $PM_{2.5}$, which is ubiquitous in urban air. PAHs have carcinogenic, teratogenic and mutagenic toxicity, being able to cause cancers of skin, bladder, lungs and kidneys via inhalation pathway. For the purpose of clarifying health risk and source characteristics of $PM_{2.5}$-bound PAHs in Beijing, we collected 218 daily $PM_{2.5}$ samples at one site in the center urban district for one year mainly in 2016 and measured the concentrations of 16 USEPA priority PAHs by gas chromatography/mass spectrometric system (GC/MS). Lifetime excess cancer risk (ECR) of the residents due to inhalation exposure to PAHs was evaluated by multiplying Benzo(*a*)pyrene (BaP) equivalent concentrations (BaP_{eq}) by unit risk value provided by USEPA (IUR = 6×10^{-7} per ng/m^3). Source apportionment of PAHs was conducted by principal component analysis/absolute principal component scores (PCA/APCS) and potential source contribution function (PSCF) based on air mass backward trajectory. The result showed that annual mean concentration of total $PM_{2.5}$-bound PAHs (TPAHs) were 82.49 ± 138.10 ng/m^3. In heating season from 15 November to 15 March, the mean TPAHs were 194.80 ± 178.75 ng/m^3, nearly 10-fold of that in non-heating season (19.92 ± 30.83 ng/m^3). Carcinogenic risk was evaluated as 9.56×10^{-6} when the residents exposed to PAHs the whole year. This risk was 23.82×10^{-6} in heating season, nearly 15-fold of that in the non-heating season (1.62×10^{-6}). Carcinogenic risk of either the whole year or two seasons were all higher than the USEPA guideline value (1×10^{-6}), which needs to be concerned. Three sources, i.e. vehicle emission, coal combustion and wood combustion & petroleum volatile source were identified, contributing 61.66%, 28.19% and 10.1% to the atmospheric PAHs, respectively. PAHs may be transported with $PM_{2.5}$ through long-distance from Shanxi, Shaanxi, Henan, western part of Hebei and middle of Inner Mongolia provinces, areas belonging to arid and semi-arid region with a majority of coal production bases and industrial parks in China.

MP193 Utilizing climate projection data to assess population vulnerability to nutrient contamination in private well water from future flooding

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Private well users are vulnerable to climate change impacts on source waters. However, climate projection data are often not available in the form needed for adaptation planning. We present an approach for utilizing projection data to characterize future flood impacts on private wells and potential exposures to nitrate contamination. A GIS-based overlay model was developed for Minnesota based on precipitation projections, well locations, land use, and demographics. The model helped to derive a composite index of future vulnerability for populations on private wells specific to June when rainfall is heaviest and nitrate fertilizers are applied to cropland. We describe the process of adjusting projection data to reflect precipitation extremes using available data and basic measures of statistical distribution. Results were cast in a qualitative frame of flood risk by comparing to past rainfall events that led to federal disaster declarations at the county level. Our approach for estimating precipitation extremes produced values similar to those at the 95th percentile using historical data, demonstrating that this method is reliable for estimating future extremes using projection data. June rainfall associated with past flood events was approximately 8.2 inches. By mid-century, most counties will experience June extremes (i.e. 95th percentile) at or above 8.2 inches. Counties with the highest projected extremes also have a majority of susceptible wells. Our composite index suggests that even where nitrate applications are high, overall vulnerability can be low if wells are not within a floodplain and population growth will be minimal. Our approach for estimating future flood risk to private wells is accessible, transparent, and therefore transferable to other jurisdictions. As a case study of scenario-planning, this approach is an example of how professionals outside of climatology can and should apply climate projection data to support the design and implementation of adaptation strategies within their own sectors.

MP194 The mixture toxicity of antibiotics to E. coli: Both the target proteins of chemicals and their effective combined concentration take immense effect

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We human beings and a class of organisms in the environment have been exposed to a variety of mixture contaminants all the time, which could pose potential ecological risk. So it is very necessary to give more focus on the mixture toxicity study of multiple chemicals. In the past few decades, antibiotics have been widely and immoderately used in all walks of life such as pharmaceutical industry and husbandry, are they drawing increasing concern at home and abroad. However, research about the mixture toxicity of antibiotics especially their mechanism is very little. So in this paper we selected sulfonamides as the main target, investigated the effects and mechanism of mixture toxicity between sulfonamides and some other different kinds of antibiotics, such as trimethoprim, quinolones, penicillin V potassium, tetracyclines, etc. Then we tried to explain the results from the respect of both qualitative and quantitative. The individual and mixture toxicity of several antibiotics were determined using *Escherichia coli* as a target organism in our study. The results show that different effects occur between different antibiotics. For example, antagonistic effects arise between sulfonamides and tetracyclines, synergistic effects behave between sulfonamides and trimethoprim, while additive effects happen between sulfonamides and quinolones. Moreover, the toxicity mechanism of binary mixtures is also discussed based on the approach of Quantitative Structure Activity Relationships (QSARs) and molecular docking and the results reveal that the differences of mixture toxicity depend not only the target proteins of individual chemicals but also on their effective combined concentration. This work is beneficial for evaluating the ecological risk of antibiotics and provides insight into understanding the mechanism of binary mixtures.

Remediation/Restoration

MP195 Adsorption of crystal violet from quaternary basic dye mixture onto a sawdust-based adsorbent

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Wastewaters from textile and other dye-using industries usually contain more than one dye. In such an aqueous matrix therefore, the adsorptive characteristics of a dye species are likely to be affected by the presence of the other dye species in the medium. The removal of crystal violet from aqueous solution by sulphuric acid-treated sawdust of locust bean tree, and the influence of malachite green, methylene blue and rhodamine B on its adsorption from binary, ternary, and quaternary dye systems were studied. The combined effect of mixture components and process parameters on the adsorption was studied and optimized using response surface methodology. The adsorbent was characterized and the experimental data obtained were fitted to different kinetics and isotherm models. The experimental results were analyzed using the Analysis of variance (ANOVA) statistical concept. The optimum contact time, pH, adsorbent dose and temperature were found to be 275.10 min, 9.94, 0.99 g and 60 ^oC respectively for the maximum decolorisation of 68.39 mg/L CV (97.2%). A linear model was obtained for the decolorization process through this design. The experimental values obtained were in good agreement with predicted values, and the model developed was highly significant, with correlation coefficient of 0.985. The adsorption in all the dye systems investigated followed Freundlich isotherm, and the maximum monolayer adsorption capacity was between 18.87 - 24.39 mg/g, depending on the composition of the adsorbate matrix. The adsorption kinetics was well described by the pseudo-second order model ($R^2 > 0.95$). All the eight adsorbate systems investigated were endothermic (?H positive; 35.30 to 43.60 $KJmol^{-1}$), thermodynamically feasible (?G: -2.30 to -6.13 $KJmol^{-1}$) and had increased entropy.

MP196 Biotic and abiotic degradation of CVOCs in clay till after controlled emplacement of guar stabilized ZVI using direct-push technology jet injection

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Remediation of chlorinated volatile organic compounds (CVOCs) in low-permeability formations, such as clay till, is often difficult due to poor contact between reagents and contaminants trapped in the matrix. This problem is particularly common in glaciated regions such as the northern United States, parts of Europe and most of Canada. Direct-Push Technology Jet Injection (DPT JI) is a method that may be able to overcome this challenge by combining high pressure jetting (69,000 kPa) and controlled hydraulic fracturing to emplace amendments into low-permeability formations. In this study, DPT JI was used to emplace 50 tonnes of guar stabilized zero valent iron (ZVI) into a 700 m^2 clay till trichloroethene (TCE) source zone in Nivå, Denmark (the Site) for in situ chemical reduction (ISCR) of CVOCs. During the first two years of performance monitoring TCE decreased from 31.8 kg at baseline to 5.6 kg, a reduction of 82%; total CVOC mass decreased from 32.8 kg to 18.2 kg (as TCE), a reduction of 45%. Reductions in soil CVOCs are typically coincident with ZVI filled fractures, and zones of treatment around individual fractures appear to be expanding over time. ISCR of CVOCs using ZVI occurs through biotic and abiotic processes. Abiotic dechlorination happens when contaminants come into direct contact with ZVI and is predominantly through beta-elimination. Biotic dechlorination results from the overall reducing conditions created by ZVI in groundwater and is predominantly through hydrogenolysis. Determining the relative contribution of abiotic and biotic degradation processes is critical to optimizing treatment performance. Low groundwater TCE (< 11.5 µmol/L) and high cis-dichloroethene (cDCE) (>50 µmol/L) following two years of treatment suggest significant biotic reductive dechlorination is occurring; cDCE is the first intermediate in the biotic hydrogenolysis pathway. The detection of elevated non-volatile organic carbon in source zone groundwater suggests that guar degradation has been slower than expected and is continuing to serve as an electron donor supporting biotic reductive dechlorination of CVOCs. However, the detection of elevated ethene and ethane concentrations (>50 µmol/L combined), without large increases in vinyl chloride, suggests abiotic degradation is also occurring within the source area. As guar continues to degrade, ZVI reactivity is expected to increase resulting in a shift to abiotic dechlorination as the dominant degradation pathway.

MP197 Blue Valley Iron Oxide Substrate and Sand Mixture Capacity to Remove Phosphorus from Solution

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Runoff from greenhouse and nursery operations contributes to phosphorus (P) loading into natural waters, which can result in eutrophication of surface waters. Removal of P from runoff via sorption to a substrate (e.g., calcined clay, crushed brick, and blast furnace slag) is a well-established mechanism for P removal from municipal and agricultural wastewater. Residuals produced by neutralization of acid mine drainage (AMD) are a promising medium for P sorption. The overall objective of this study was to determine the effectiveness of AMD iron oxides for use in P filtration of greenhouse runoff. A series of batch sorption experiments was conducted to determine the P sorption capacity of Blue Valley iron oxides (BVIO), an AMD product. The P sorption capacity of mixtures of silica sand and BVIO were quantified at 1:1 (volume: volume, BVIO: sand), 1:3, and 1:9 in a laboratory scale study. For each iron oxide-sand mixture, 3 g of substrate was added to 50mL centrifuge tubes, and assigned one of five concentrations of P (0.5, 1.0, 10.0, 50.0, 100 mg/L P, added as KH_2PO_4. The sorption capacity of each BVIO:sand mixture was calculated. Phosphorus sorption kinetics and the influence of pH on sorption capacity to BVIO:sand mixtures were also quantified for each treatment. Preliminary results indicate a correlation between the amount of BVIO in each treatment and the amount of phosphorus sorbed. The 1:1 treatment achieved a P sorption capacity of 18,248 mg P/kg substrate, while the sorption capacities of the 1:3 and 1:9 treatments were 15,337 and 11,919 mg P/kg substrate, respectively. These sorption results are comparable to P sorption capacities reported for other acid mine drainage iron oxide sources, supporting the viability of this product for use in P filtration technology. Results from this study will inform design of a pilot-scale filter for sorbing P from greenhouse runoff.

MP198 Chemical modification of lignocellulosic materials for the retention of heavy metals present in water

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Among the heavy metals discharged into the environment as a product of industrial activity is chromium. Chromium is an active metal in the environment. Cr (VI) is very toxic and has great mobility while Cr (III) is non-toxic at low concentrations (Selvi et al., 2001). The main sources of chromium contamination are industrial activities such as electroplating, leather tanning, wood preservation, alloy preparation, mining, cement, dyeing, electroplating, painting and photographic material (Selvi et al., 2001). Different chemical processes have been developed to eliminate the chromium present in wastewater. The most commonly used methods for reducing the concentration of chromium in aqueous solutions are ion exchange on polymer resins (Leyva et al., 2010), coagulation-flocculation, adsorption on coal activated and reduction/chemical precipitation/sedimentation. This last method is the most used but it is very inefficient, since a lot of sludge is produced and the precipitated chrome can not be recovered, and this makes it very expensive. In this sense, adsorbents of tannin origin obtained from the tree bark are a clear alternative to retention of chromiun from wasterwater. An adsorbent was synthesized through tannin polymerization obtained from Pinus bark extracts. The best conditions of temperature, concentration, stir among others were taken into account to obtain the adsorbent. Tannin-derived rigid polymers were very effective adsorbents for the removal chromiun from aqueous solution.

MP199 Comparative study of polystyrene and polyethylene degradation by insect larvae

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In recent years, microbial plastic degradation has become a huge area of interest. In most cases, plastic disposal occurs by recycling, conversion to a different product or chemical degradation. Until very recently, polyethylene (PE) required certain additives to make it degradable. To find a more prudent way to deal with this challenge, researchers have concentrated their efforts on bioremediation. Recently, Yang et al. (2014) showed that polyethylene was degraded by the gut microbial community of *Plodia interpunctella* (larvae of Indianmeal moths). While the gut microbiota of these larvae may possess plastic-degrading capabilities, the process itself is unclear. The types of microorganisms involved and any changes in regulation during PE degradation is still unknown. We recently completed the metagenomic study of polystyrene (PS) degradation by *Tenebrio molitor* (larvae of the Mealworm beetle). We showed that the native gut microbial community of *Tenebrio* itself can degrade polystyrene without much change to its community composition or structure. However, there were certain changes seen in microbial abundances. The most abundant species found were *Enterococcus*, *Akkermansia*, *Bacteroidales*, *Lactococcus* and *Vagococcus* to name a few. In PS-fed *Tenebrio*, *Enterococcus* sp. was shown to have a significantly decreased abundance while that of *Vagococcus* was increased. The current study reports and compares between the metagenomic diversity and identification of major gut microbial species present in *Plodia* fed with Polyethylene (as their only food source) compared to *Plodia* fed a normal diet of bran. Microbial DNA extracted from these larvae was sequenced by targeting the v3 and v4 regions of 16S rRNA. Qiime and Usearch analysis pipelines helped identify the microbial species along with statistical tests to determine any significant changes in abundances. From the previous PS study, there are abundance changes between the 2 diets and similar changes are commented upon here as well. Comparative studies between gut microbial abundance of PE-fed *Plodia* and metagenomic diversity of PS-fed *Tenebrio* allow for identification of similar microbial species possessing polymer-degrading capabilities. Results from this study contribute to understanding of the functionality of this mixed community that enables it to degrade PE successfully. Applications for plastic recycling, one of the most intractable problems of human society today are expected deliverables from this study.

MP200 Effects of Surfactants and Mycobacterium vanbaalenii PYR-1 Bioaugmentation on Polycyclic Aromatic Hydrocarbon Mineralization

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants that have potential mutagenic, carcinogenic, and teratogenic properties. Bioremediation has been recognized as an efficient and versatile approach to remediate PAH-contaminated soil sites. However, the biodegradability of PAHs is limited by their bioavailability to microorganisms in the soil porewater fraction. To expedite biodegradation, the addition of synthetic surfactants or biosurfactants at the critical micelle concentration (CMC) has been used to enhance the bioavailability of PAHs. Additionally, the bioaugmentation of PAH-degrading microorganisms can be implemented to increase PAH biodegradation. To evaluate the effects of Brij-35 surfactant and rhamnolipid biosurfactant at three concentrations (0.1X CMC, 1.0X CMC, and 10X CMC) and the bioaugmentation of *Mycobacterium vanbaalenii* PYR-1 in PAH-contaminated soil, ^{14}C-pyrene mineralization was assessed in two soils with varying physiochemical properties. The addition of Brij-35 surfactant at all three concentrations resulted in increased ^{14}C-pyrene mineralization after 50 days incubation by indigenous microbe populations in the sandy loam and clay soil compared to the unamended and rhamnolipid-amended soil treatments. The bioaugmentation of *M. vanbaalenii* PYR-1 had an immediate impact on ^{14}C-pyrene biodegradation in both soils, resulting in approximately 60% mineralization after 9 days. Additionally, both surfactant amendments did not enhance the mineralization by *M. vanbaalenii* PYR-1 as compared to the unamended bioaugmented treatments. The addition of rhamnolipid biosurfactant delayed ^{14}C-pyrene mineralization in the bioaugmented soil treatments in a dose-dependent manner. It appears that the rhamnolipid biosurfactant acted as a more favorable carbon source compared to ^{14}C-pyrene and was preferentially degraded by the PAH-degrading microorganisms. Overall, the results of this study provide beneficial insights towards the abiotic and biotic processes as well as their complex and sensitive interactions in the bioremediation of soils contaminated with PAHs.

MP201 Monitoring at and Future of Pig's Eye Landfill and Pig's Eye Lake

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The Minnesota Pollution Control Agency (MPCA) is conducting a feasibility study of Pigs Eye Landfill and Pig's Eye Lake. The Landfill is the largest (212 acres) un-permitted landfill in Minnesota, situated in the Mississippi River floodplain near downtown St. Paul. Battle Creek flows through the central portion of the Landfill and discharges to 500-acre Pig's Eye Lake. Major flooding occurs every three to five years in the wetland adjacent to the Creek and Lake. The Metropolitan Council and U.S. Army Corps of Engineers express interest in developing the Landfill and Lake for recreational use and improve the Lake ecosystem. The Landfill operated as a dump without permits from the mid-1950s to 1985, accepting 8.2 million cubic yards of municipal, commercial, and industrial wastes. The MPCA placed the Landfill on the State Superfund Permanent List of Priorities in 1989, and environmental monitoring started in 1992. The MPCA completed several Response Actions at the Site including consolidation and waste relocation, removal of over 200 drums from the Landfill and along the Creek, reworking the toe of the landfill and lower reach of the Creek to pull back waste material, and erosion control on the shoreline of the Creek and Lake. The Landfill was capped in phases (2001 – 2005) to a 2-foot cover using soils from local construction projects. The soil piles were sampled and compared to 2005

MPCA Soil Reference Values (SRVs). Metals in the cover soil piles exceed current (2016) SRVs. A Long-Term Maintenance and Monitoring Plan developed in 2006 included annual monitoring of metals groundwater and surface water in the Creek and Lake to confirm no migration of contaminants. Perfluorinated compounds (PFCs) and metals were identified in exceedance of MPCA established surface water criteria in groundwater and surface water. Pigs Eye Lake sediment has been sampled by the MPCA and other agencies from 1994 through 2016. Metals, PAHs, PCBs and PFCs in sediment exceed MPCA Sediment Quality Targets. A sediment risk assessment was conducted using analytical data, toxicity testing data, and a fish tissue PFC data. Metals in sediment pose unacceptable risk to benthic organisms. The remedial action objectives being pursued by MPCA are: Reduce risk to the benthic invertebrate community due to exposure to contaminants in sediment. Reduce potential human health risk from direct contact with landfill cap materials. Reduce the migration of PFCs in groundwater to Pig's Eye Lake.

MP202 PAH contaminations and recoveries in the sandy versus muddy sediment coastlines after the Hebei Spirit oil spill

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After the collision between the oil tanker Hebei Spirit and a barge carrying a crane, approximately 10,900 tons of Middle East crude oil was released 10 km off the west coast of Korea in December 2007. Total 438 sediment samples were collected to assess the spatiotemporal trends of PAH contaminations and its recoveries, from December of 2007 through May of 2016. PAH concentrations in sediments measured immediately after the spill ranged from 3.2 ng g^{-1} to 71,200 ng g^{-1}, with an average of 3,800 ng g^{-1}. Increases in PAH concentrations were observed at stations 7 through 23, which were heavily oiled due to tidal currents and northwesterly wind that transported the spilled oil to these locations. Mean and maximum PAH concentrations decreased drastically from 3,800 to 88.5 ng g^{-1} and 71,200 to 1,700 ng g^{-1}, respectively, four months after the spill. PAH concentrations highly fluctuated for the first one year after the spill and then decreased slowly to background levels. Reduction rate of PAH concentration was much faster at the sandy beaches (half-life = 43.3 d) than in the muddy sites (half-life = 693 d). In muddy sediments, low attenuation due to low flushing rate in the mostly anaerobic sediment possibly contributed the persistence of PAHs. By May of 2016 (~8.5 yrs after the spill), mean and maximum PAH concentrations decreased by ~ 50 and 500 times, respectively, compared to the initial contamination levels. Compositional profile of PAHs in sediment collected immediately after the spill was similar to that of the spilled source oil having a greater fraction of low molecular weight PAHs. Alkylated PAHs also far exceeded unsubstituted parent PAHs. As the amount of the spilled oil diminished, the preferential loss of low molecular weight PAHs and the decrease of alkyl to parent PAH ratios were clearly noticed.

MP203 Phytoremediation and restoration potential of red and blue beam laser photo stimulated Alfalfa (Medicago sativa L.) plants

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Alfalfa possesses important characteristics for phytoremediation that includes fast growth, high biomass, and extensive tap root with a robust rhizosphere system. We conducted a greenhouse experiment to observe the phytoremediation and restoration potential of alfalfa grown in three different sandy loam soil contaminated by diesel (2% w/w) weathered for 180 days. Three replicates for both laser stimulated and non-stimulated plants were maintained in cone-tainers. The effect of laser photostimulation on alfalfa germination, biomass yield, and plant mortality was measured for 7 weeks. Destructive sampling of the plants and soil was done at day 0, 21, 35, 42 and 49 for quantification of remaining diesel, plant growth, biomass and microbial community diversity in the rhizosphere region. Germination rates were not affected by 180 seconds of red beam followed by 30 seconds of blue beam laser photostimulation every 72 hours, as both laser stimulated and non-stimulated alfalfa seeds germinated at 34% and 37% respectively. Biomass yield was also not affected by laser photostimulation, however, the lateral root development was significantly enhanced in laser stimulated plants.

MP204 Profiling of plants at petroleum contaminated site for phytoremediation

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The lack of data that establishes the type of plant to be used in the phytoremediation of petroleum-hydrocarbons-contaminated sites brought about the need for this study. The study aimed to identify native plants growing around a petroleum-hydrocarbon-contaminated site in Umuahia, Nigeria that have the ability to phytoremediate soil TPH in a tropical monsoon climate region. Twenty-eight native plant species of different families growing around the PAH-contaminated site due to vandalisation of the petroleum pipeline were sampled. Most of the plants demonstrated the ability to grow in a high concentration of the total petroleum hydrocarbon, which is an indication that they can be used for phytoremediation of a petroleum-hydrocarbon-contaminated soil. *Chromolaena odorata, Aspilla africana, Chloris babata, Pasparlum vaginatum, Bryophylum pinnatum, Paspalum scrobiculatum, Cosmos bupinnatus, Eragrostis atrovilens, Cyperus rotundus, Uvaria chamae* all demonstrated phytoremediation characteristics in the contaminated site. Those characteristics included the ability to survive the obnoxious nature of TPH contamination and subsequently uptake a greater part of the contaminant. By using bioaccumulation coefficient (BAC) as a module for phytoremediation, plants were screened based on their BAC values. From this study, *chromolaena odorata, Aspilla Africanus* and *Uvaria chamae* were selected for induced phytodegradation of PAHs studies.

MP205 Role of Agricultural Waste in the Removal of Heavy Metals and Hormones in multi-contaminant wastewater

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In many parts of the world, water scarcity is problematic. The ever-increasing population has increased the food demand. Therefore, wastewater is considered as an alternative option for crops irrigation. However, wastewater treatment plants (WWTPs) was found to be inefficient for the removal of emerging contaminants. Therefore, crops irrigation with wastewater could pose a risk on the human health. The aim of this study is to investigate the efficiency of biochar produced from agricultural waste i.e. rice straw and wheat straw in the removal of heavy metals and female hormones in wastewater. Sorption/desorption experiments were carried out to assess the removal of heavy metals (Cd, Cr, Cu, Fe, Pb and Zn) and hormones (estrone, 17β estradiol, and progesterone) in wastewater. The removal efficiency of rice straw biochar produced at 550 ^{0}C biochar was (100%) for the three hormones; indicating a significant difference with wheat straw biochar. For heavy metals, the removal efficiency ranged from 68 % to 100% for rice straw and wheat straw. Desorption experiments showed that less amounts of heavy metals were released from rice straw compared to wheat biochar. Therefore, using biochar produced form rice straw and wheat straw could be a low cost and effective technique to reduce the contamination in the crops irrigated with wastewater.

MP206 Solving the Puzzle of a Dynamic Shoreline: Investigation of a Former Firing Range on the Shores of Lake Erie

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A Remedial Investigation was completed (2012-2014) for eight miles of the Lake Erie shoreline near Port Clinton, Ohio, a former impact area for munitions testing for the U.S. Army. Both munitions constituents (MC) contamination and munitions and explosives of concern (MEC) hazards existed. Numerous challenges faced the site investigation team due to the dynamic nature of the shoreline, which is subject to tides, wave action, and varying water depths. The shoreline is intermittently covered with water at varying depths or is dry for day, weeks, or months at a time. What media should be sampled? How would a sufficient number of representative samples be obtained to evaluate the human and ecological receptors? Where and when should sampling take place? The site investigation and risk assessment team worked with the stakeholders (U.S. Army National Guard Directorate, Ohio EPA, and U.S. Army Corps of Engineers) to come up with a feasible and representative sampling plan. A sampling plan was developed that incorporated random, biased, and incremental sampling of surface and subsurface sediment. The human health risk assessment was conducted for workers, residents, recreational beach users, fishers, and hunters and the ecological risk assessment was conducted for both terrestrial and aquatic receptors. The risk assessments took into account that there are many possible sources of contaminants in Lake Erie sediments and surface water. Ultimately, the sampling was sufficient to satisfy all stakeholders and to determine that limited risks related to past Army activities exist for both human and ecological receptors at this complex lake environment.

Aquatic Toxicology and Ecology – Endocrine Disruptors

MP208 6-OH-BDE-47 increases coiling behavior and apoptosis in zebrafish embryos (Daniorerio)

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Polybrominated diphenyl ethers (PBDEs) are a group of brominated flame retardants that are ubiquitously detected in the environment, and which are associated with adverse health outcomes in several research studies. 6-OH-BDE-47 is a potential metabolite of 2,2',4,4'-tetrabromodiphenyl ether (BDE-47), and there has been considerable focus to its developmental neurotoxicity and endocrine disrupting properties. In this study, we found that exposure to 6-OH-BDE-47 (50-100 nM) from 4 hpf to 26 hpf in zebrafish embryos results in 2.3 to 3.5 times higher coiling behavior and significant increases in apoptotic cells in the brain. These effects were rescued by over-expression of Thyroidhormone receptor β (THRβ) mRNA. Moreover, 100 nM 6-OH-BDE-47 significantly decreased Tryptophan hydroxylase 2 (TPH2) mRNA expression (6 times, $P< 0.05$), the rate-limiting enzyme in the biosynthesis of Serotonin (5-HT). This research indicates that 6-OH-BDE-47 may affect nervous system development in the early life development of zebrafish embryos, and that the reduction of TPH2 mRNA may play a role in neurotoxicity zebrafish. Furthermore, this work suggests that 6-OH-BDE-47 affects thyroid hormone regulation through THRβ, which in turn affects the neurological behavior of the embryos. Moreover, this study also showed that coiling behavior could potentially be used to investigate neurological toxicity in early stage zebrafish embryos.

MP209 Bisphenol A alters the cardiovascular response to hypoxia in Danio rerio embryos

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The purpose of this study was to determine if the cardiovascular response to hypoxia was altered by the presence of bisphenol A (BPA) in *Danio rerio* embryos. It was expected that BPA exposure would affect cardiovascular parameters during hypoxia more than normoxia due to an interaction between BPA and the hypoxia-inducible factor pathway. Cardiovascular parameters were measured in vivo using video microscopy and digital motion analysis. We demonstrate that BPA exposure has a minimal effect during normoxia but can severely affect the cardiovascular system during a hypoxic event. Co-exposure to BPA and hypoxia lead to severe bradycardia and reduced cardiac output at higher concentration of BPA. This was accompanied by developmental defects, decreased developmental rate, and high mortality that were absent in normoxia treatments. With lower concentrations, some decreases in cardiac parameters were observed but were ameliorated post exposure. Blood flow, measured as red blood cell velocity, however was 31 % lower in hypoxia co-exposure treatments than normoxia in caudal arteries. After 6-7 days post exposure, velocity decreased further resulting in a 63% difference between hypoxia and normoxia with 0.001 µg/L of BPA. While cardiac parameters were sensitive to higher concentrations, vascular parameters experienced larger decreases at lower concentrations and several days post exposure. Our results show for the first time that BPA exposure alters the cardiovascular system during hypoxia more so than normoxia. We also demonstrate that this effect varies with concentration and time of exposure. More research is needed to determine how co-exposure affects larval fish as they proceed through development and ultimately reach adulthood.

MP210 Ubiquitous contaminants of emerging concerns mandate endocrine examinations with various receptors of diverse wild animals

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Contaminants of emerging concerns (CECs) have been detected ubiquitously in aquatic environments, and their endocrine-disrupting effects are worrisome. We have identified typical CECs in rivers associated with agricultural and urban land use in the Great Lakes watershed. A mixture of agricultural CECs (AG) was assembled to mimic the environmentally occurring compounds and consisted of 8 chemicals, while an urban CEC mixture (UB) contained 11 chemicals including the known estrogenic compounds, bisphenol-A, estrone and nonylphenol in both mixtures. According to the EPA ToxCast database, AG and UB at an environmental relevant concentration were estimated to account for 13% and 6% estrogenicity of estradiol-17β (E_2), respectively. Although toxicities of these compounds have been tested on various assays in model species, their long-term effects need to be evaluated using endocrine endpoints in diverse species. Two isoforms of estrogen receptor (Esr) cloned from fathead minnow, bluegill sunfish, American alligator and human were separately expressed in human embryonic kidney 293T cells, and their transcriptional activities were detected using estrogen-response elements and luciferase reporter gene to quantify estrogenicities of agricultural and urban CECs by comparing with serial dilution of E_2. Two isoforms of Esrs cloned from 4 species differently responded to AG and UB mixtures. Human ESR1 was the most sensitive to AG based on their estrogenicities, while minnow Esr1 was the least sensitive to AG. Bluegill Esr1 was the most sensitive to UB based on their estrogenicities, whereas BG Esr2a was the least sensitive UB in receptors we tested. Even at an environmental relevant concentration, human ESRs, alligator ESR2 and bluegill Esr1 were significantly activated by both AG and UB. Moreover, 100 times concentrated UB hyper-stimulated minnow Esr1 and alligator ESR2 at 30-49% higher than the maximum induction of E_2. Therefore, human, alligator and bluegill estrogenic signals could be disrupted in both agricultural and urban habitats. Minnow would be the most resistant to CECs-exposure at both habitats among the four species tested. These results indicate that efficacious receptors and species differ between CECs mixture, and further endocrine studies of CECs are required utilizing a variety of receptors cloned from diverse threatened and endangered species.

Revisiting Global Climate Change and Its Influence on the Practice of Environmental Toxicology and Chemistry

TP001 Why the continued grumbling about SETAC's lack of involvement with the global climate change research program?

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In previous articles, we and others have argued the global climate change research community has overlooked the issue of climate change and chemical contaminants, and we suggested that one way to address this oversight is for SETAC and the membership to become more involved. Our past efforts to highlight this issue include a Pellston-style workshop in 2011, publications in ET&C and IEAM, and a recent ET&C Focus article. In the latter, we note a continued lack of focus on contaminants in the recent Intergovernmental Panel on Climate Change Reports (2013 and 2014), other than those chemicals known or suspected to be radiative forcing, such as the greenhouse gases carbon dioxide and methane. Exposure to legacy and new chemicals, coupled with the changes likely to result from global climate change, could have unexpected negative consequences for terrestrial and aquatic organisms, ecosystem services and humans. We highlight some specific actions that SETAC leadership and members could take to address this lack of attention.

TP002 Riverine Sources of Mercury to Aquatic and Terrestrial Ecological food Webs: Effects of Remediation and Climate Change

J. Flanders, AECOM Environment; R.G. Stahl, E. I. du Pont de Nemours and Company / Corp Remediation

The South River is a high gradient stream with mercury (Hg) impacts downstream of a former acetate flake and yarn facility in Waynesboro, VA. The consumption of methylmercury (MeHg) in potential dietary items is the largest source of risk to humans and ecological receptors in the watershed; this presentation considers the potentially antagonistic effects of climate change and remediation on the concentration of MeHg in biota and risk. Aquatic invertebrates are important routes of exposure for fish and other aquatic and terrestrial receptors. MeHg enters the food web through the consumption of organic matter by larval invertebrates - the MeHg content of the organic matter is controlled in part by inorganic Hg (IHg) concentration in water/sediment, water temperature and surface water discharge. Based on this conceptual understanding of exposure, remediation will focus on control of IHg sources (e.g., erosion of Hg impacted soil) to the aquatic environment. Over time, remediation should reduce loads to the river, and the concentration of IHg should decline due to dilution by lower IHg-content suspended sediment transported to the river from upstream of the historic mercury source, release of fine-grained sediment from the streambed, and reduced bioavailability of IHg in sediment or on particles. Climate change and urbanization could affect the rate of recovery. For example, the potential increases in rainfall intensity predicted by climate models or urbanization may affect the relative sediment transport rate from the upper watershed. Increased temperatures associated with climate change are predicted, but their effects on Hg methylation rates are uncertain. Increased MeHg concentrations are associated with surface water temperatures above 12^{o}C in the South River, suggesting that conditions favorable for methylation could persist for longer periods throughout the year in the future. However, there is some evidence that an optimal temperature range for methylating bacteria exists in the South River, resulting in lower net methylation during the warmest periods. In summary, the management of large-scale (e.g., watershed scale) Hg-impacted legacy sites is complicated by the scale of the impact and the potentially long time required for measurable improvement following remediation. Predictions about the rate of improvement are further complicated by the uncertainty posed by climate change and associated changes in large-scale environmental characteristics.

Fate, Toxicology or Risk Assessment of Materials of Interest to the Military

TP003 Contact Transfer of a Highly Toxic Chemical Warfare Agent from Contaminated Grass

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Field investigations suffer from an inability to adequately control inherently unstable ambient variables. Studies conducted under controlled environmental conditions allow focus on critical parameter outcomes. Investigations of chemical–plant interaction using actively growing plants must maintain normal plant physiology to yield results applicable to the field. We successfully developed innovative research methods for investigating chemical–plant interaction, sustaining living healthy grass plants (E. crus-galli) within a Surety Hood for extended periods of time. Grass is the most prevalent type of higher plant worldwide, and natural distribution of E. crus-galli is one of the largest. Traditional lighting in plant growth chambers typically involves balancing heat-loads with chilling units too cumbersome for most laboratory hoods. To alleviate lighting heat-load we installed a controlled array of improved light-emitting diodes (LED) within a Surety Hood to supply sufficient levels of high-quality photosynthetically active radiation (PAR). Surety Hood conditions were: PAR illumination 300–350µmoles cm^2 sec^{-1}, 16h-light/8h-dark; 22°C ±2; relative humidity 50% ±10; airflow 2.41kph ±0.14. We disseminated 1µL droplets of nerve agent O-ethyl-S-(2-diisopropylaminoethyl) methyl phosphonothioate (VX) onto individual mature grass leaves which were then removed at intervals, and 3-layers of Army Combat Uniform (ACU) swatches were placed atop each contaminated leaf, with the bottom swatch in direct contact with the contaminated leaf surface. The set of ACU layers was then covered with a 0.64cm-thick Plexiglas disk to equally distribute force resulting from 1kg standard mass centrally-atop the disk for 10min. Total proportions of VX transferred at 0.017, 0.25, 0.5, 1, 4h post-dissemination were 71, 5, 0.8, 0.3, 0.1%, respectively, of disseminated-VX per leaf; traces of VX were detected in uppermost 3rd-layer of ACU at 0.017 and 0.25h post-dissemination. Our experimental data: 1) comport with historic field studies of VX on sod (grass), when effects of uncontrolled rainfall and outdoor temperatures are taken into account; 2) provide critical parameter input for predictive models; 3) are reliable determinations for comparison to predictive model outcomes; 4) provide information for decision-making affecting Soldiers, First Responders, and public safety. These methods are useful for investigating chemical–plant interaction for additional classes of chemicals and plants.

TP004 Characterization of Chemical Warfare Agent VX on Contaminated Grass and Wash-off Factors Following Rain Events

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We successfully developed innovative methods for growing and maintaining actively growing plants in a surety hood, disseminating chemical warfare agent (CWA) O-Ethyl-S-(2-diisoproylaminoethyl) methyl phosphonothiolate (VX) droplets onto actively growing grass foliage (*Echinochloa crus-galli*), and evaluating the visual appearance and spread of the VX droplets. Persistence, absorption, and evaporation of CWA droplets on foliage depends on many factors, including rainfall. We characterized and quantified VX droplets on grass foliage, and determined wash-off factors for VX following rain events. In separate experiments, 1µL or 3µL VX droplets were dispensed onto leaves of living intact plants. Each droplet was photographed on foliage at timed intervals and the image was converted to surface area (SA) by imaging software. Initially, the droplet appeared as a transparent wet area that spread with time. After 1h, both the 1 µL and 3 µL droplets ceased to spread, and leaf tissue within the droplet spread area appeared darkened. After 24h, the affected leaf tissue appeared necrotic (light tan in color). The mean SA (mm^2) of the affected tissues was 132, 192, 163, 135, and 142 at 0.017(1min), 0.05, 1, 4, and 24h, respectively for the 1 µL droplet; and 166, 301, 303, 278, and 274 at 0.05, 1, 4, 24, and 48h for the 3 µL droplet. Necrosis and desiccation of affected tissues accounted for decreased SA at the latter time points. Using droplet SA values, we experimentally established wash-off coefficients (k_w) for the 1 and 3 µL VX droplets at 0.017, 1, and 4h post-dissemination. A 10mm rain event at 0.017h washed off 95% (1µL droplets) and 83% (3µL droplets) of the VX, respectively. At 1h, a 10mm rain event washed off 0.03 and 0.5%, of 1 and 3µL VX droplets, respectively. The k_w values for 1 and 3 µL droplets, respectively, at 0.017h post-dissemination are 0.095 and 0.083 mm^{-1}. At 1 and 4h post-dissemination, k_w values were approximately 3 orders of magnitude less than those at 0.017h. Based on these results, the majority of the VX on grass can be washed off by a moderate rainfall within minutes post-dissemination. The hazard is then transferred to the water and soil. These results are the first to quantitatively characterize CWA droplets on leaf surfaces and provide critical inputs for predictive models for VX. Information provided herein can be used by Warfighters and first responders to substantially accelerate both assessment and mitigation following release of VX.

TP005 Development of a high resolution mass spectrometry (HRMS) spectral library of poly- and perfluoroalkyl substances (PFASs)

J. Brown, Colorado School of Mines / Civil and Environmental Engineering; S.C. Roberts, Sciex, Redwood City, CA; K. Barzen-Hanson, Cornell University / Biological and Environmental Engineering; S. Mass, A. Maydanov, Colorado School of Mines / Civil and Environmental Engineering; A.E. Robel, J. Field, Oregon State University / Environmental and Molecular Toxicology; C.P. Higgins, Colorado School of Mines / Civil Environmental Engineering

The complex chemistry, persistence, and potentially significant ecological and human exposure and toxicity from poly- and perfluoroalkyl substances (PFASs) necessitate the expansion of tools available to evaluate risk, determine the extent and nature of contamination, and mitigate hazards. While the current regulatory framework is focused on perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) and to some extent perfluorobutanoic acid (PFBA), perfluorobutane sulfonate (PFBS), and perfluorohexane sulfonate (PFHxS), the potential formation of these chemicals (and other PFASs) from precursors are a major focus of the Department of Defense SERDP/ESTCP environmental research programs. To address these needs, an extensive extracted ion chromatrogram (XIC) list of approximately 1,400 identified and theoretically-predicted PFASs as well as a HRMS spectral library of PFASs was developed. As HRMS is a widely available technology and is already being used for SERDP/ESTCP PFAS research, this approach provides a logical path for the development and validation of a broadly applicable PFAS forensic tool useful for source and plume delineation, identification of a broad range of PFASs in the environment, and evaluation of mitigation strategies.

TP006 A Low-Cost, Rapid-Screening Assay for Analysis of Per- and Polyfluoroalkyl Substances (PFASs)

D. Bogdan, AECOM / Environment; G.N. Peaslee, Notre Dame / Physics; D. Lunderberg, Hope College / Chemistry and Physics; U.K. Vedagiri, AECOM / Design and Consulting

Per- and polyfluoroalkyl substances (PFASs) have been used in many industries, i.e., aerospace, apparel, auto, construction, chemicals and pharmaceuticals, electronics, energy, healthcare, oil and gas, and semiconductors. The term PFASs is attributed to a very large class of chemicals composed of many thousand molecules with different physical, chemical, and biological properties from one another (Buck et al., 2011). Available analytical methods through commercial labs can typically only analyze < 35 PFASs and university laboratories more than 240 PFASs (quantitative or qualitative) (Barzen-Hanson et al., 2017). However, toxicological information of many PFASs is not currently available, making the results hard to interpret and to decide what are the appropriate actions. A novel analytical method has been developed and validated to measure total fluorine present in aqueous samples, such as groundwater contaminated with PFASs. The method involves pre-concentration by solid-phase extraction disks, and subsequent identification and quantification of the total F present on the surface of the disk by Particle-Induced Gamma-ray Emission (PIGE) spectroscopy. This ion beam analysis technique requires only minutes per sample to achieve detection limits on the order of micrograms per liter, depending on the volume of sample pre-concentrated. The assay provides a measure of the total organic fluorine content that exists as weakly anionic compounds, as they are extracted efficiently while non-anionic compounds are not. Inorganic fluoride ions can be easily eluted off the disk. This analytical tool was also used to analyze PFASs present in paperboard, wrappers, and textiles (Ritter et al., 2017; Schaider et al., 2017). PIGE can also detect and measure PFASs applied to surfaces of wide variety of products. The PIGE results could be used for a variety of purposes including, but not limited to, screening tool, vulnerability assessment, conceptual site model development, or environmental monitoring.

TP007 Literature Review of Reported BCF and BAF Values for Perfluorooctane Sulfonic Acid (PFOS) in Fish and the Implications on Treatment Cost

D. Bogdan, AECOM / Environment; U.K. Vedagiri, AECOM / Design and Consulting

Direct and/or indirect releases of perfluorooctane sulfonic acid (PFOS) to the surface waters resulted in fish advisories due to the PFOS concentrations that have been found in fish fillets. The calculated bioconcentration factors (BCFs) and bioaccumulation factors (BAFs), either presented in literature or calculated at different sites, have been used by some states to develop surface water criterion. The surface water criterion is used as allowable discharge concentration or ambient surface water concentration, which in the end has a direct impact on the cost of removing PFOS and other per- and polyfluoroalkyl substances (PFASs) from the environment. The first interlab comparison conducted in 2005 showed up to 125% relative standard deviations (RSD) for analysis of PFOS in standards, fish tissue, and water (van Leeuwen *et al.*, 2006). During a recent proficiency testing a total of 126 laboratories from Australia and around the world were invited to participate and only 24 submitted results. In the absence of a standardize analytical method, it was found that laboratories are using different sample pretreatment, extraction technique, extraction solvents, clean-up, and equipment for the analysis of PFOS in fish samples (NMI, 2016). From of total of 9 laboratories only 5 submitted satisfactory results for all three matrixes: fish, soil, and water. A literature review of BCFs and BAFs will be presented along with the implications on treatment cost. Recommendations for fish sampling in order to prevent cross contamination along with current limitations and considerations for BAF calculations will also be presented.

TP009 Factors controlling human exposures to PAHs in soil and methods for assessing bioavailability

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Several studies reported in the literature suggest that interactions between PAHs and soil diminish PAH bioavailability and thus reduce risk from incidental ingestion of PAH- contaminated soil. This presentation will provide an overview of a broad, multi-organization research effort to explore the influence of soil composition, PAH concentration, and PAH source material type on PAH bioavailability to humans, using an approach capable of measuring uptake at low, environmentally-relevant PAH concentrations (down to 1 ppm or less). This research program, funded by the Strategic Environmental Research and Development Program (SERDP), evaluated the chemical controls on the oral bioavailability and dermal absorption of PAHs in soil. Major findings of the effort are that the relative oral bioavailability of benzo(a)pyrene (BaP) was observed to be strongly dependent on PAH source material and ranged from about 20% (PAHs in black carbon matrices) to 90% (PAHs in fuel oil). Dermal absorption of BaP was not observed to be dependent on soil composition for the four soils tested (different PAH source materials were not tested) and was lower than USEPA's current default value for dermal absorption of BaP. Results from this study also indicate that rapid and inexpensive in vitro methods show good promise for predicting the oral RBA of PAHs from soil. The implications of these data for human health risk assessment will be discussed, along with their potential impacts on soil cleanup goals. This poster provides a summary of the broad scale investigation, components of which are being presented in other SETAC sessions.

TP010 Determination of Ecotoxicological Effects of Insensitive Munitions Compounds in Soil

R.G. Kuperman, US Edgewood Chemical Biological Center / Molecular Toxicology; R. Checkai, US Army Edgewood Chemical Biological Ctr / Molecular Toxicology / Environmental; M. Simini, US Army Edgewood CB Center / Environmental Toxicology; M.V. Haley, US Army Edgewood Chemical Biological Center / Molecular Toxicology Environmental

The United States military services are developing and evaluating several insensitive munition compounds (IMs) for future weapon systems to replace present munitions that contain highly sensitive explosives, such as RDX and TNT. Among these compounds are the IMs 2,4-dinitroanisole (DNAN) and 3-nitro-1,2,4-trizole-5-one (NTO), intended for use as components in several insensitive munition explosive compositions (IMXs). Ecotoxicological data are needed to address a clear gap in current knowledge of the potential risks of release of DNAN or NTO into soil. To fill the existing data gaps for DNAN and NTO, we designed our studies to meet regulatory requirements for developing Ecological Soil Screening Levels (Eco-SSL) for use in screening-level ecological risk assessment, and Species Sensitivity Distributions (SSD) for each of these IMs to determine specific hazardous concentrations of DNAN and NTO for use in establishing Soil Cleanup Values (SCVs). Ecotoxicological data will be developed for each IM weathered-and-aged in soil to more closely approximate soil microenvironments representative of field conditions, and to reflect the bioavailability of the individual IMs and their transformation/degradation products. Studies will be conducted with a soil type that supports high relative bioavailability of IM compounds. Eco-SSLs and SCVs developed from this research can help minimize the future costs of site characterization and cleanup when used by site managers as part of their science-based decision making tools to assess the exposure risks at each site, and when appropriate, develop remedial actions and SCVs that ensure the management of sites as sustainable resources.

TP011 Ecological risk assessment of munitions constituents in aquatic environments

G.R. Lotufo, US Army Engineer Research and Development Center / Environmental Laboratory; M. Ballentine, US Army Engineer Research and Development Center; R. George, US Navy, SPAWAR SSC; G. Rosen, SPAWAR Systems Center Pacific

Historically, manufacturing, open burning or detonation, improper disposal and dumping, as well as testing, training and combat operations, have resulted in the release of munitions constituents (MC) to terrestrial and aquatic environments. Contamination associated with MC has been reported world-wide for a wide variety of environments, including near-shore and off-shore ocean, wetlands, rivers, and lakes. Contamination results from nonpoint sources (e.g., terrestrial runoff) or point sources (e.g., wastewater treatment plant discharge, corroded underwater munitions). Compilation and examination of available data indicate that concentrations of MC (e.g., trinitrotoluene [TNT] and RDX [hexahydro-1,3,5-trinitro-1,3,5-triazine] in water and sediment were largely below detection or were relatively low (e.g., parts per trillion or parts per billion), with higher concentrations being highly localized and typically near a point source. These findings were in accordance with predictive modeling and with studies of the fate of MC in aquatic environments. Available toxicity data derived for a variety of freshwater and marine species was compiled and used to derive interim water quality criteria and protective values derived from species sensitivity distributions. Toxicity varied widely across a diversity of MC and species. For most aquatic sites, MC contamination in sediment and in the water-column seem to present low risk to the resident biota.

TP012 Acute and Short-Term Chronic Toxicity of 3-nitro-1,2,4-triazol-5-one (NTO) to Ceriodaphnia dubia, Daphnia pulex, D. magna and Pimephales promelas

D.A. Pillard, TRE Environmental Strategies / Environmental Toxicology; R.B. Naddy, TRE Environmental Strategies

The compound 3-nitro-1,2,4-triazol-5-one (Nitrotriazalone or NTO) belongs to a group of new energetic materials, known as Insensitive Munitions eXplosives (IMX), which are being developed to replace conventional munitions explosive fills such as TNT. These IMX compounds are more stable and much less prone to unintended detonation when exposed to threat on the battlefield or in the logistics supply chain. Acute and short-term chronic (STC) studies were conducted with neutralized (pH-adjusted to ~7.5) NTO using freshwater species that are commonly used in laboratory aquatic toxicity testing: *Ceriodaphnia dubia, Daphnia pulex* and *Daphnia magna* (Crustacea: Cladocera) and the fathead minnow, *Pimephales promelas* (Cyprinidae). The purpose of these studies was to evaluate inter-species sensitivities to NTO and provide a basis for comparison to the toxicity of more traditional energetics. Survival was evaluated in all tests; sub-lethal endpoints were reproduction in *C. dubia* and growth (dry weight) in *P. promelas* and *D. magna*. Test procedures generally followed USEPA guidance for whole effluent toxicity (WET) testing. The invertebrates were more sensitive to NTO than the fish in both acute and chronic studies, with 48-h LC50s ranging from 838 to 1,179 mg/L of NTO. The 96-h LC50 for *P. promelas* was over 6,300 mg/L. Invertebrates were also more sensitive in the STC tests. While the lowest No Observed Effect Concentration (NOEC) was found for *D. magna* (38 mg/L, based on growth), *C. dubia* presented the lowest IC25 at 67 mg/L NTO, based on reproduction. The IC25 for *P. promelas*, based on growth, was 390 mg/L. Although there is a limited amount of NTO toxicity data available, particularly on these species, the current and other published data for *C. dubia* suggest it is one of the more sensitive freshwater aquatic species tested thus far. This presentation will contrast and compare data from these studies to other data available in the literature.

TP013 Ecotoxicity of the insensitive munitions compound 3-nitro-1,2,4-triazol-5-one (NTO) and its reduced metabolite 3-amino-1,2,4-triazol-5-one (ATO)

C.L. Madeira, University of Arizona / Chemical and Environmental Engineering; S.L. Levine, Monsanto Company / Chemical and Environmental Engineering; M.T. Simonich, Oregon State University / Environmental and Molecular Toxicology, The Sinnhuber Aquatic Research Laboratory and the Environmental Health Sciences Center; R.L. Tanguay, Oregon State University / Sinnhuber Aquatic Research Laboratory and the Environmental Molecular Toxicology; J. Chorover, University of Arizona / Soil Water and Environmental Science; R. Sierra-Alvarez, University of Arizona / Chemical and Environmental Engineering

The insensitive munitions compound 3-nitro-1,2,4-triazol-5-one (NTO) has been used by the army as replacements for conventional explosives. After reaching the soil, NTO was reported to undergo microbial reduction to 3-amino-1,2,4-triazol-5-one (ATO), which is recalcitrant in saturated soils. As the use of NTO becomes widespread, there is more concern about the toxicity of this compound. In this study, the toxicity of NTO and ATO is assessed towards three microbial targets: methanogenic archaea, aerobic heterotrophs, and the marine bacterium *Aliivibrio fischeri* (Microtox assay). The acute toxicity of ATO to the microcrustacean

Daphnia magna was evaluated. Lastly, zebrafish embryos (*Danio rerio*) were exposed to NTO and ATO, and the behavioral and developmental effects to 22 endpoints were evaluated. NTO was notably more inhibitory than ATO to methanogens (IC_{50} = 1.0 mM, > 62.8 mM, respectively). However, ATO had a greater inhibitory impact on aerobic heterotrophs (IC_{50} = 31.0 mM). Similar toxicity effects were found for both compounds in the Microtox assay, which was not very sensitive to NTO or ATO. In the *Daphnia magna* assay, the mortality of 50% of the organisms was observed for the ATO concentration of 0.4 mM, while NTO was reported to cause the mortality of 50% of the organisms at a concentration of 3.5 mM in an assay conducted with *Ceriodaphnia dubia* as the target organism. Exposure of zebrafish embryo to NTO or ATO did not have developmental effects on any of the endpoints tested. However, abnormalities in the swimming behavior were observed at concentrations as low as 7.5 µM for both NTO and ATO. The results indicate that the reductive biotransformation of NTO could enhance or lower its toxicity according to the target organism.

TP014 Evidence of Anaerobic Coupling Reactions between Reduced Intermediates of 2,4-Dinitroanisole (DNAN)

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2,4-Dinitroanisole (DNAN) is a nitroaromatic compound that is gradually replacing trinitrotoluene (TNT) in military explosives. DNAN, an insensitive munition, is safer than TNT because it is less prone to unintentional detonation. Consequently, it does not fully combust, and leaves behind a significant amount of residue on firing ranges. This residue may then enter the soil and groundwater during rainfall events. Anaerobic bacteria in waterlogged soils, for example, are known to reduce nitroaromatics to their corresponding aromatic amines. The widely accepted fate is that these aromatic amines react with oxidized soil minerals and/or O_2 to form radicals that (co)polymerize with each other and/or phenolic moieties present in the soil. Preliminary research has shown that anaerobic conditions lead nitroaromatics to a different fate. We hypothesize that reduced intermediates of nitroaromatics, in particular nitrosoaromatics and aromatic amines, couple with each other via nucleophilic substitution to form azo-bonded dimers and oligomers. These azo compounds may be more toxic than the original nitroaromatics. In the absence of O_2, it is also possible that amino groups of reduced nitroaromatics react with quinone moieties in soil humus to form adducts. This effectively immobilizes these toxic reduced intermediates and coupling products. The objective of this study is to elucidate the coupling reactions between reduced intermediates of DNAN and gain further insight into the fate of nitroaromatic compounds in anaerobic environments. 4-Nitroanisole was chosen as a model compound for the more complex DNAN. To study the overall process of biological reduction and chemical coupling, 4-nitroanisole was spiked gradually into incubations of anaerobic granular sludge. The formation of the azo dimer 4,4'-dimethoxyazobenzene was confirmed by liquid chromatography, UV-Vis spectroscopy, and mass spectrometry. However, the azo bond was not stable in the sludge incubations, and the dimer did not accumulate. Other, uncharacterized suspected coupling products formed and accumulated in this experiment, although the major intermediate was 4-aminoanisole.

TP015 Acute and chronic effects and toxicokinetics of the insensitive munitions constituent methylnitroguanidine

M. Ballentine, US Army Engineer Research and Development Center; G.R. Lotufo, US Army Engineer Research and Development Center / Environmental Laboratory; J.P. Chappell, SOL Engineering / Environmental Laboratory; J.A. Brame, US Army Engineer Research and Development Center

The Army continues to strive to develop high pay-off munitions that are safer for both the warfighter and the environment. The focus of this study was to evaluate the effects of 1-methyl, 3-nitroguanidine (MeNQ) on 7 aquatic species (juvenile *Pimephales promelas*, *Rana pipens*, *Daphnia pulex*, *Hyalella azteca*, *Chironomus dilutus*, *Lumbriculus variegatus*, and *Hydra littoralis*). MeNQ is a constituent of novel insensitive munitions formulations that are of interest to the military, and is the methylated version of nitroguanidine (NQ) and therefore understanding its hazard is relevant. Acute lethality of MeNQ was evaluated for all above species in exposures up to 3000 mg/L, and chronic toxicity was evaluated using juvenile *P. promelas*, *D. pulex*, and *H. azteca*. A MeNQ toxicokinetic experiment was completed on juvenile *P. promelas* including an uptake and depuration phase allowing for the calculation of uptake and elimination rates. Acute toxicity was low for all species, with significant reduction in survival occurring only for *D. pulex*. Sublethal effects were observed as significantly decreased reproduction at 188 mg/L for *D. pulex* and 1500 mg/L for *H. azteca*. The acute toxicity experiments were used to calculate acute freshwater water quality criteria using a species sensitivity distribution. Finally, photo activation of MeNQ resulted in increased acute lethality to juvenile *P. promelas*, as mean survival was 15% and 100% in photo-activated and non-photo activated 600 mg/L treatment, respectively. Information on the uptake and elimination kinetics, as well as on the bioconcentration potential of MeNQ will be presented.

TP016 Field validation of a passive sampling device for assessment of conventional munitions constituents in aquatic environments

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Historically, manufacturing, open burning or detonation, improper disposal and dumping, as well as testing, training and combat operations have resulted in the release of munitions constituents (MC) to terrestrial and aquatic environments. In underwater environments, unexploded ordnance (UXO), low order detonations, or discarded military munitions, may be sources of MC exposure, including trinitrotoluene (TNT), degradation products of TNT (e.g. aminodinitrotoluenes and diaminonitrotoluenes), and trinitrohexahydro-s-triazine (RDX) to the water column or surface sediments. However, there are limited reports associated with underwater MC. Detected concentrations have generally been reported as very low (e.g. part per trillion; ng/L concentrations) on localized scales. It is further recognized that underwater MC exposure may fluctuate over time based on site hydrodynamics and multiple factors associated with the condition of individual munitions, and their contents. Our team previously demonstrated the value of use of Polar Organic Chemical Integrative Samplers (POCIS) for detection and semi-quantitative estimation of MC in an estuary followed by strategic placement of samplers around a known quantity of the explosive fill Composition B (Rosen et al. 2016). Here, we report on the first known use of POCIS at a former U.S. Naval training range (Vieques, Puerto Rico), where high densities of UXO are present in some bays, but where little to no knowledge of their propensity to be leaking MC exists. A total of 30 POCIS field canisters were deployed by munitions response divers in Bahia Salina del Sur, 15 of which were within 12 inches of potentially breached munitions, for three weeks. Discrete water samples were collected adjacent to the same munitions during POCIS deployment and recovery operations. All 30 samplers were recovered. Of these, four and one samples were above the quantitation limits for RDX (range 4-13 ng/L) and TNT (5.3 µg/L), respectively. Detection with grab samples were generally similar in frequency and magnitude to those from POCIS. It was noted that collection and solid-phase extraction of larger water volumes (e.g. 10-20 L) instead of traditional 1 L water grab samples may have made traditional and passive sampling more comparable. The POCIS and grab sampling results, however, suggest that even the highest MC concentrations observed at the site are substantially lower than those expected to be hazardous to the most sensitive aquatic species and ecotoxicological endpoints.

TP017 Ecological Risk Assessment at a Canadian Military Test Site

J. Olson, GHD

An ecological risk assessment (ERA) was conducted for a heavily used range at a military test site located in eastern Canada. The study area consists of several ranges that have been used for decades for many purposes, including small arms firing range, experimental test range, rocket range, and open burn/open detonation. The contaminants of concern included a wide range of military constituents such as explosives, propellants, oxidizers, fillers, metals, and combustion by-products, as well as petroleum hydrocarbons and volatile organic compounds associated with a former waste disposal site within the study area. Because of the presence of varied natural habitats bordering the ranges, the receptors of concern included a wide range of terrestrial and aquatic receptors including plants, soil invertebrates, benthic invertebrates, fish, amphibians, reptiles, birds, and mammals. The risks to each of these categories of receptors was assessed through a weight of evidence approach using several lines of evidence such as soil, water and sediment chemistry, toxicity tests, a food chain model and an assessment of plant health. In the case of terrestrial vegetation, birds, mammals and amphibians, the lines of evidence indicated the absence of unacceptable risks caused by contamination on the site. Some possible risks were identified for soil invertebrates due to the toxicity results for soils in areas near potential sources of contamination. Possible risks to benthic invertebrates were identified in the lake on the site from contamination by ammonia, lead and benzo(b+j+k) fluoranthenes in sediment. The risks to aquatic life varied between the different water bodies on the site. Water from the lake was found to have significant toxicity in tests using Fathead minnow (*Pimephales promelas*) and *Ceriodaphnia dubia*. However, the few toxic effects observed with respect to water from the streams and the river bordering the study area could not be conclusively linked to contamination from the site. In conclusion, few risks were identified to ecological receptors within the study area though there is some uncertainty as to the risks to certain receptors. Recommendations aimed at reducing this uncertainty are presented.

TP018 Using the Trident Probe to Delineate Groundwater Discharge Areas Containing Elevated Nutrient Concentrations Along a Lagoon Shoreline

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Historical water quality surveys conducted at an impaired water body in southern California, the Santa Margarita Lagoon – Oceanside (CA), indicated the potential for excess nutrients associated with groundwater discharges originating upland from previously operational agricultural fields located just north of the lagoon. Multiple site investigation phases (2010 – 2014) involving pore water sampling were conducted to identify likely nutrient venting zones along the Santa Margarita Lagoon shoreline and offshore areas. Each phase of the investigation involved the collection of pore water for the initial measurement of water quality parameters (i.e. conductivity, temperature, total dissolved solids [TDS], oxidation-reduction potential [ORP], and pH) and then followed by dissolved nitrogen and phosphorus analyses of a subset of samples. All thru-out the sequence of these investigations, a US Navy, Space and Naval Warfare [SPAWAR] Systems Center Pacific (SSC-PAC) -developed Coastal Contaminant Migration Monitoring (CCMM) assessment tool, the Trident Probe, was utilized. The Trident Probe is a direct-push, integrated conductivity-temperature (CT) sensor and groundwater-surface water interface (GSI) sampler used for groundwater discharge zone identification and sampling. During this multi-year investigation, sub-surface sampling for interstitial pore waters were collected from numerous locations (120+) along the northern shoreline, offshore, and upland areas of the lagoon. Preliminary water quality screening measurements from the Trident [and verified by a hand-held meter] of the pore waters subsequently identified three (3) potential discharge areas which showed signs (lower conductivities) of freshwater inputs from groundwater. Dissolved nutrient analysis of the pore water sample splits from these locations showed elevated nitrate-nitrogen levels (10-110 mg/L). The laboratory results confirmed that these were the areas where the nutrient-rich, groundwater was discharging into the lagoon. A summary of the Trident Probe technology, methods, and capabilities etc. as well as a subset of the study data will be presented.

Chemical, Biological and Instrumental Methods for Detecting Harmful Algae Blooms and Their Natural Toxins

TP019 Measurement of Blue-Green Algal Toxins in Air and Water at Tainter Lake, Dunn County, WI

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Blue-green algal blooms occur every year in Tainter Lake, a eutrophic flowage lake on the Red Cedar River in Dunn County, WI. Blue-green algae can produce several types of toxins, including microcystins. In 2004, microcystins were measured in the water of Tainter Lake at concentrations as high as 1,400 ug/L. The current USEPA Draft Recreational Ambient Water Quality Criteria/Swimming Advisory for microcystins is 4 ug/L. Given the severe blooms on this lake, and reports of human health complaints by residents living near this lake, there is a need to determine if inhalation may be a viable route of exposure. The purpose of the current study was to determine concentrations of microcystins (microcystin-LA, microcystin-LR, microcystin-RR, microcystin-YR), cylindrospermopsins (cylindrospermopsin, deoxycylindrospermopsin), and anatoxins (anatoxin-A, homoanatoxin-A) in lake water and in nearshore air. We collected samples from four different sites on one date in August, 2015 and from three different sites on two dates in August, 2016. Duplicate water samples and air samples were collected from each site for anlaysis of toxins by high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). Concentrations of microcystins were also determined in select air samples using enzyme-linked immunosorbent assay (ELISA). Concentrations of chlorophyll-a and dissolved phosphorus were measured at each site. Chlorophyll-a concentrations ranged from 28 to 135,000 ug/L. Dissolved phosphorus concentrations ranged from 30 to 136 ug/L. Microcystin-LR was detected in every water sample collected. Highest concentrations measured for microcystin-LA, microcystin-LR, microcystin-RR and microcystin-YR were 636, 518, 536, and 89.9 ug/L respectively. No cylindrospermopsins or anatoxins were detected in the water samples. No toxins were detected in the air samples in 2015 when analyzed by HPLC-MS/MS. However, when two of those air samples were analyzed by ELISA (total microcystins, lower detection limit), one sample contained 0.18 ug/L microcystins. No toxins were detected in the air samples in 2016 when analyzed by ELISA. In the future, air samples will be collected after the bloom has started to decay, when cells may release more toxins to the air. Data from this study strengthens the need for a fully-implemented TMDL (total maximum daily load) plan for phosphorus in the Red Cedar River watershed.

TP020 Harmful algal bloom smart device application: Using image analysis and machine learning techniques for classification of harmful algal blooms

M. Waters, Northern Kentucky University / Mathematics Statistics; J.M. Lazorchak, J. Allen, USEPA / Office of Research and Development

Northern Kentucky University and the USEPA Office of Research Development in Cincinnati Agency are collaborating to develop a harmful algal bloom detection algorithm that estimates the presence of cyanobacteria in freshwater systems by image analysis. Green and blue-green algae exhibit different Hue-Saturation-Value color histograms in digital photographs. These differences are exploited by machine learning techniques to train a smart device (cellular phone, tablet, or similar) to detect the presence of cyanobacteria in a small surface portion of a freshwater system. The Harmful Algal Bloom Classification Application (HAB APP) has been field tested and verified to classify both green and blue-green algae. Specifically, the APP has been tested on several small streams and ponds, correctly classifying green algal blooms and has been tested on the Ohio River, correctly classifying blue-green algae in the 636-mile cyanobacteria bloom in summer 2015. The application is being tested via fixed camera monitoring stations and optimized at several locations along the Ohio River and in Lake Harsha, a 22,000-acre reservoir which supplies six million gallons per day of drinking water to the Ohio county in which it lies and is a source of many recreational activities, including swimming, boating, and fishing. The presence will be verified by other detection instruments and in vitro by agency scientists and hysteresis techniques will be used to monitor the presence of cyanobacteria on a periodic (e.g., daily, seasonally) basis at the monitoring stations. Further, the APP is being extended to classify harmful algae microscopically at the genus level using a convolutional neural network approach.

TP021 Assessing the Solid Phase Adsorption Toxin Tracking (SPATT) bag method for the Determination of HAB Biotoxin Concentrations in Estuarine Ecosystems

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Harmful algal blooms (HABs) are the overgrowths of algae that cause harm by toxin production or by biomass accumulation. HABs are influenced by sunlight, carbon-dioxide, and nutrients. Other sources include temperature, salinity, wind, water-depth, and grazing predators. The goal of this study is to determine HAB biotoxin concentrations in Apalachicola National Estuarine Research Reserve (ANERR) and Grand Bay National Estuarine Research Reserve (GNERR) and to determine the correlations to water column nutrient concentrations. Solid Phase Adsorption Tracking (SPATT) bags will assess the amount of dissolved biotoxin levels of domoic acid in seawater. Nutrient and other data will be obtained from the NERR's System-Wide Monitoring Program (SWMP). Dissolved biotoxin extracts will be measured using bioassays. A spiking protocol will measure the efficiency of the porous resin, where salinity, humic acid, and domoic acid concentrations will be tested in deionized water, artificial salt water, and estuarine collected water. Using 1L Erlenmeyer flask, SPATT bags will be exposed to the various treatments for 2 weeks and ELISA will measure toxin uptake, while spectrophotometry will measure humic acid levels. Changes in the Earth's climate will impact the production of HABs. This study is important in sharing more information on some factors that influences HAB production. This framework will add value to the greater scheme of maintaining healthy oceans. [key words: HABs, SPATT bags, nutrients]

TP022 Lake Winnebago 20 Years Later: Revisiting the Cyanotoxin Methods and Results from Raw and Finished Waters at Four Wisconsin Drinking Water Facilities

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Freshwater harmful algal blooms (HAB) and the toxins they may produce are of increasing concern in recreational and drinking waters due to their potential public health risk. Nearly 20 years ago the Wisconsin State Laboratory of Hygiene and the Wisconsin Department of Natural Resources conducted a two-year study of the occurrence of the cyanotoxin microcystin in raw and finished waters from five drinking water treatment facilities located on Rainbow Lake and Lake Winnebago, Wisconsin (USA). In 1998 & 1999 a total of 1026 raw, finished, and treatment train water samples from the drinking water treatment facilities were collected and tested for microcystins using the first of its kind (at the time) enzyme-linked immunosorbent assay (ELISA) for cyanotoxins. Over the subsequent years there have been several major HAB events in the United States including the 2014 major bloom in Western Lake Erie that forced the City of Toledo to shut-down their drinking water supply for several days. There have also been major advancements and improvements to drinking water treatment technologies utilized at the facilities located on Lake Winnebago, including the addition of ozone and ultraviolet light processes. The methods used to detect cyanobacteria-related toxins, including improvements to the original ELISA test, have also advanced significantly within the last 20 years. These bloom events and new innovations prompted us to revisit the 1998/1999 study to ensure microcystins were still being effectively removed at the original four Lake Winnebago drinking water treatment facilities. We will present the methodology and cyanotoxin results from the 1998/1999 study as well as data from the > 500 samples collected eighteen years later in the summer and fall of 2016.

TP023 A Connection Between Algal Bloom Toxicity and Organic Matter in Ohio Waters

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Harmful algal blooms (HABs) are a direct health threat to the public that use Ohio waterbodies for recreation and drinking water. The prediction of upcoming blooms and the measurement of bloom toxicity are critical to managing this public health problem. Current bloom forecasting models, which rely heavily on nutrient tracking, have had inconsistent results. MODIS, ELISA and qPCR can measure current blooms, but are unable to predict them. There is a need for an inexpensive, accurate and proactive method of predicting the formation and toxicity of HABs that will provide water management authorities advanced warning of impending bloom conditions. The objective of this research is to determine if developing toxic algal blooms are associated with changes in bulk dissolved organic matter (DOM) composition. We have adopted a fluorescence spectroscopic approach to monitoring the characteristics of DOM from bloom prone waters weekly during the bloom season, typically June until September. Further, we have collected phytoplankton monthly from bloom prone waters to determine bloom toxicity using Thamnotoxkit-F. Results indicate that the growth of toxic cyanobacteria algal blooms is preceded by the development of a unique fluorescing DOM moiety. Bloom toxicity is associated with the abundance of this DOM moiety suggesting that changes in DOM composition portend the development of toxic HABs in the aquatic systems examined here. If this shift in DOM characteristics is ubiquitous and directly related the HAB development, water treatment plants could use a fluorescence probe tuned to the specific fluorescence characteristics of the DOM moiety of interest to predict the severity and toxicity of impending algal blooms before they form.

TP024 Algal Blooms, Anatoxin-a Production, and Potential Effects on Human, Animal, and Ecosystem Health

V. Christensen, USGS; R.P. Maki, National Park Service / Voyageurs National Park; E.A. Stelzer, USGS / Ohio Microbiology Laboratory

Harmful algal blooms (HABs) containing toxin-producing cyanobacteria are a growing worldwide concern because they can negatively affect humans, animals, and ecosystems. Microcystin is a widely monitored cyanobacterial toxin; however, other toxins associated with HABs are not well characterized in the environment. Anatoxin-a, one of the most potent algal toxins, is not widely monitored despite its known acute effects. We collected water samples from Kabetogama Lake in northern Minnesota from June through September in 2016-17 for laboratory analyses including phytoplankton identification, microcystin, and anatoxin-a. In addition, quantitative polymerase chain reaction analyses were used to examine the cyanobacteria's DNA and determine potential for toxin production. Microcystin concentrations were lower than previous years; however, anatoxin-a genes were present. Additional testing will indicate whether anatoxin-a was produced by the HABs in Kabetogama Lake and at what concentrations. An important finding related to public safety is that anatoxin-a producing strains were present before the blooms were visible. The occurrence of anatoxin-a in Minnesota waters may have implications for lake ecosystems and chronic health effects due to repeated exposure.

TP025 Generating ecotoxicity information on microcystins and prymnesins: A different approach

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There is a lack of information for estimating safe levels for aquatic life concerning the toxicity of natural toxins produced by cyanobacteria and algae. There are a number of reviews that have indicated that the toxicity of microcystins to daphnia and zebrafish is not as great as it is to humans and mammals. Current literature indicates the LC50s for Microcystin LR range from 1 – 21 mg/L. There is even less ecotoxicity information available for prymnesin which is produced from the estuarine golden algae *Prymnesium parvum* that has invaded freshwater systems in the U.S., even though there have been numerous fish kills recorded in inland Texas lakes and blooms in freshwater systems in 10 other states. Given the uncertainty of standards for bacteria and algal toxins, their cost of using them to conduct acute and chronic toxicity tests and their potential impurities, a new approach is proposed using pure cultures and ambient bloom samples. Herrera, Echeverri and Ferrao-Filho (2015) conducted acute and chronic toxicity tests on several different cladoceran species using lyophilized phytoplankton samples collected from hyrdroelectric/drinking water reservoirs in Brazil. They found that reservoir samples with higher microcystin contents were the most toxic ones and that different cladocerans had different sensitivities to microcystin. In this study we have taken a similar approach but we used laboratory cultures of toxic *Microcystis aeruginosa* and *P parvum*. Each culture was centrifuged to remove each species at its peak production of toxin, then resuspended in moderately hard water followed by a freeze/thaw step 3 times at -80 C similar to procedures used for ELISA methods. Initial chemistry results of the *M aeruginosa* cultures indicate that a 100-x diluted sample had 3.8 ug/L MC-LR, 1.1 ug/L D-Asp3-MC-LR, and 1 ug/L MC-LA or a total microcystin concentration of 600 ug/L. *Ceriodaphnia dubia*, *Daphnia magna*, *Hyalella azteca* and larval *Pimephales promelas* were exposed to a similar extract. A similar procedure was also used on lake water samples collected during peak bloom condition. The exposure method and comparison of results of the lab culture and lake samples will be presented.

TP026 Overview of USEPA Office of Research and Development's research on analysis and monitoring in fresh and coastal/estuarine environments

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Several factors are contributing to the development of the "perfect" Harmful algal Bloom (HAB) storm. Elevated temperatures and changes in precipitation, changes in population demographics, agricultural land use linked to nitrogen loading increases, and an aging water treatment infrastructure all combine to increase the probability of toxins being present in consumers' taps. In the past several years there have been several notable HAB events in a number of states. August 2014 a Lake Erie HAB event occurred that impacted the city of Toledo's drinking water supply with elevated levels of microcystin (≥1 μg/L). In November 2015 California issued a warning along its coastline to consumers of Dungeness and Rock Crabs of contamination of a Cyanobacteria toxin known the domoic acid. In 2015 an algal bloom occurred along the Ohio River spanning over 680 miles. In 2016 Lake Okeechobee experienced an algal bloom that impacted the Caloosahatchee and St. Lucie River systems as well. Increases in salinity are also having adverse ecosystem impacts by creating blooms of invasive toxic algae. The most problematic of these is the marine invasive *Prymnesium parvum* (golden algae) where blooms have been documented in at least 11 states. As a result of the human, ecological, and economic impacts of HABs, USEPA has established a research program to address issues related to the detection, quantification and monitoring of algal blooms in freshwater and marine systems. This research has several objectives: develop new or refine existing chemical, instrument, biological methods and camera apps for the detection of HABs and their toxins; test such methods in field studies in both HAB and non HAB environments; determine the method(s) that can be best used as early warning systems for the detection of HABs and their toxins. We will present the latest research being conducted that include chemical detection of toxins in water and fish tissues, molecular detection methods (eDNA, PCR, qPCR) of HAB species and the genes responsible for toxin production, online toxicity monitors (OTM), in vitro methods for detection of toxicity, flow cytometry, mass spectrometric, and microscopic approaches for phyto/zooplankton identification, Phone apps for HABs early warning and Cyanobacteria and algal identification, and advanced instrumental and hyperspectral image analysis approaches.

Adverse Effects of Chemicals on the Microbiome

TP027 Di-2-(ethylhexyl) phthalate (DEHP) effect on intestinal microbiome

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Phthalates are a ubiquitous chemical class used as plasticizers in plastic products. These chemicals are not covalently bound to the polymer matrix and can leach out of products. Phthalates are found in relatively high concentration in indoor air, surface waters, sediments, and perhaps most alarmingly, in municipal drinking water. Phthalates, namely DEHP (di-2-(ethylhexyl) phthalate), are now recognized as an emerging environmental contaminant of concern for human health due to their ubiquitous and widespread use and for their potential to disrupt metabolic processes. Recent studies suggest that shifts in the gut microbiome is one of the key factor that effects metabolism because this results in impaired intake of nutrients and hormone balance in the gut. The goal of this study is to determine whether phthalates adversely affect the zebrafish (*Danio rerio*) intestinal microbial community composition and its diversity. This may lead to phthalate-induced metabolic disruption. To simulate chronic exposure of DEHP, we exposed the fish to 3 ppm DEHP via food for 30 and 60 days. Fish were fed once a day with commercial food mixed with DEHP. Fish were dissected and the content of gut was collected. After the microbial DNA isolation, we measured the V1-V3 variable region of 16S ribosomal RNA genes using Illumina MiSeq. All sequences were de-noised using USEARCH and analyzed for chimeras using UCHIME. Sequence data were then separated into operational taxonomic units (OTUs) and annotated using the RDP classifier with GreenGenes v. 12.10 as a reference. Weighted UniFrac distances was used to determine phylogenetic relationships among samples. Bacterial sequences were annotated via mapping to either bacterial or the zebrafish genome. This poster summarize our study, where we used the latest genomic approaches to understanding gut microbiome activity in relation to human health, and gut microbial modulation influenced by long term exposure to DEHP. This research was supported by Marie Sklodowska-Curie actions no.707241- European Commission Research Program.

TP028 Low-dose exposure of Roundup/Glyphosate affects the gut microbiome of Sprague-Dawley pups

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Roundup is a broad-spectrum herbicide, considered to be nearly nontoxic to humans. Glyphosate is the active ingredient in this herbicide. However, emerging evidences argued that glyphosate may be a key contributor to the obesity and the autism epidemic in the United States, as well as to several other diseases and conditions. This proof-of-principle study examines whether low-dose exposure of Roundup/ Glyphosate to Sprague-Dawley rats from birth through adulthood will modify the composition of gut microbiome. The Roundup and pure glyphosate were administered at doses comparable to USA ADI guideline (1.75mg/kg bw/day) to the F0 female Sprague-Dawley rats starting from the 6th days of pregnancy and the fecal samples were collected at multiple time points from both F0 dams and F1 pups. The gut microbiome of 432 fecal samples was profiled by 16S ribosomal RNA gene sequencing, taxonomically assigned and assessed for diversity analysis. Metagenomics profiling revealed that the low-dose Roundup and glyphosate exposure resulted in significant and distinctive changes in overall bacterial composition in F1 pups only. Specifically, at postnatal 31 days (PND31), the LEfSe analysis showed the individual taxon relative abundance for *Bacteriodetes* (*Prevotella*) was increased while the relative abundance of *Firmicutes (Lactobacillus)* was reduced in either Roundup or glyphosate exposed F1 pups compared to water controls. This study provides initial evidence that the exposure to commonly used Glyphosate-based herbicides at doses comparable to human exposure is capable of modifying the gut microbiota in baby rats; whether these changes lead to downstream health effects requires further investigation.

TP029 The role of gut microbiota in sediment remediation by Capitella teleta

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Capitella teleta is an opportunistic marine polychaete worm that is often found at high densities after oil spills and in association with other organic pollution. *C. teleta* is known to transform polycyclic aromatic hydrocarbons (PAHs), but the mechanism of degradation is unknown. This research aims to determine if gut microbiota of *C. teleta* play a prominent role in PAH degradation. Initial sequencing of 16S rRNA from the intestinal tracts of worms from uncontaminated lab cultures revealed diverse bacteria that were unique from the sediment microbiome, and several families and genera contained PAH-degrading members. We are currently examining gut microbiota from juvenile *C. teleta* worms exposed to fluoranthene-contaminated sediment and analyzing the microbiome using amplicon sequencing and bioinformatics techniques. In this way we hope to identify important players in PAH degradation by looking for changes in bacterial composition related the fluoranthene exposure. The long term goal of this research is to use information about *C. teleta* and its gut microbiome to help to improve technologies for sediment bioremediation.

TP030 What role does the microbiome of Alitta virens play in the metabolism of sediment-associated PAHs?

E. Apazoglou, Roskilde University / Institut for Naturvidenskab og Miljø; R. Hochstein, University of Minnesota / Ecology, Evolution, and Behavior; M. Sadowsky, V. Forbes, University of Minessota; A. Palqmvist, Roskilde University / Biotechnology Institute

The marine environment and more specifically marine sediments are recipients for numerous anthropogenic hydrophobic organic contaminants, such as polyaromatic hydrocarbons (PAHs), which have the potential to act as stressors to the sediment macrofauna. In this study, we focus on the marine polychaetes *Alitta virens* a sediment-dwelling species with high ecological and economical importance. Previous studies revealed that *Alitta virens* is able to resist and accumulate high levels of PAHs from sediments while the mechanism underlying this ability remains unclear. To test the assumption that the intestinal microbiome of *A. virens* is contributing to its ability to cope with exposure to PAHs, we exposed individuals with naturally occurring intestinal microflora and individuals that had been previously treated with a mix of antibiotics in 30 µg fluoranthene /g dry weight sediment for twenty days and compared them to control (i.e., unexposed) individuals. We sampled overlying water, sediment and animal tissue at 12h, 1d, 2d, 4d, 10d and 20d after the initiation of the fluoranthene exposure. The disappearance of the parent compound, and the occurrence of selected fluoranthene metabolites were monitored by analyzing the samples by HPLC. Samples of the intestinal tissue of the individuals with natural flora were cultivated in petri dishes with marine agar and later sequenced, in order to map the alterations in microbial community composition after exposure to fluoranthene. The results document the responses of the intestinal microbiome to an exogenous chemical stressor, as well as provide insights into the relative contributions of the worm and its microbiome to PAH biotransformation . Overall the study is expected to provide a better understanding of the role of the microbiome in contaminant metabolism and detoxification.

Soil Contaminants – Fate, Bioavailability, Environmental Toxicology – Application in Ecological and Human Health Risk Assessment

TP031 Effect of Biochar on the Behavior Imidacloprid in Illinois Soil

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Imidacloprid [(*E*)-1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylideneamine] is an exceptionally potent insecticide used to control sucking and soil insects known from physiological studies to act at the nicotinic acetylcholine receptor. The application of biochar to soils has been known for some time to increase pesticide sorption and reduce efficacy. Overall, there is the assumption that increased soil sorption could reduce leaching of insecticides to groundwater, thus improving overall water quality. However, the aim of this study were to evaluate the effect of amending soils with soybean stover, sugarcane bagasse, and wood chips derived biochars or same raw feedstocks on the sorption-desorption of imidacloprid applied in a soil from Illinois, silty clay loam. We used the batch-equilibrium method to determine the concentration of [^{3}H]-imidacloprid in a pseudo-steady state with soil and biochar-soil systems at 10% (w/w). Based on the sorption-desorption coefficient (K_d) values, imidacloprid was considered relatively low sorption insecticide to the soil-unamended, with $K_{d\ sorption} = 1.66 + 0.02$ L kg^{-1} and $K_{d\ desorption} = 6.90 + 0.28$ L kg^{-1}. Sorption of imidacloprid increased up 16 times in soil-amended with biochar as compared to the soil-unamended and desorption increased up 5 times. Biochar produced from wood chips feedstock was the most effective biochar that we assessed for reducing (~93%) the aqueous herbicide concentration. Therefore, the composition of the biochar in the soil-amended can play an important role in the practical remediation by sorption-desorption of imidacloprid, because these biochars reduced the herbicide concentration.

Advances in the Development and Application of Toxicity Reference Values

TP032 Dose-Response Toxicity Analysis of PCBs - Approach to Support Cleanup Decisions

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Ecological risks were evaluated at a contaminated tidal marsh system in the northeast United States consisting of 3,800 feet of tidal waterway and 17 acres of common reed (*P. australis*) dominated marsh. Mercury, polychlorinated biphenyls (PCBs), and other chemicals of potential concern (COPCs) are present in waterway and marsh sediments at the site and the broader region. The marsh and waterways are a valuable resource actively used by a variety of wildlife. An assessment approach was designed to characterize the nature, magnitude, and likelihood of potential adverse effects due to COPC exposure. The baseline ecological risk assessment (BERA) used deterministic and probabilistic exposure modeling and dose-response toxicity analysis to inform cleanup decisions with technically defensible data evaluations that met the EPA risk assessment framework. Indirect food web exposures for wildlife were estimated by modeling chemical-specific concentrations in dietary components using combinations of site-specific and literature-based bioaccumulation models and/or measured tissue concentrations. Given varying availability of data for many exposure pathways, a series of deterministic exposure estimates were produced to focus probabilistic estimates on key pathways. Detailed review of avian toxicological data for PCBs was performed, including developing dose response relationships. Effect thresholds were established as ED20s (dose resulting in a 20 percent effect). Probabilistic models of exposure were run and were compared to ED20s to quantify likelihood and magnitude of adverse effects. Deterministic models indicated low to medium level risk within the marsh but did not inform likelihood of occurrence. However, probabilistic model results compared to the deterministic ED20s showed a low probability of adverse effects within the marsh habitat. Without a probabilistic risk evaluation, site management decisions could be based on perceived risk with low likelihood of occurrence. The use of probabilistic risk assessment, combined with dose-response analysis of toxicity data to develop risk thresholds, will result in a more informed risk management decision. The more informed decision will likely be more cost effective and may avoid active remediation that would have caused an unnecessary temporary loss of ecosystem function.

TP033 Methylmercury Toxicity Reference Values for Mammalian Wildlife Populations: Critical Review and Analysis

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We reviewed effects of chronic methylmercury exposure on non-primate mammals, with the goal of identifying toxicity reference values for the ecological risk assessment of mammalian wildlife populations. The review focused on dietary exposures in which effects on reproductive success or survival were investigated. Issues related to applying toxicity study results in ecological risk assessments were also reviewed. Relevant toxicity data were available primarily for mink and rats. Dose-response relationships and effect thresholds will be summarized. In contrast to birds, mink exhibited little difference in sensitivity between survival and reproductive endpoints, suggesting that methylmercury effects on reproductive success may be secondary to more general systemic toxicity. Important considerations in applying the available mammalian toxicity data include interspecies differences in mercury tolerance adaptations, mercury-selenium interactions, methylmercury bioaccessibility, and effects of body weight differences on dose extrapolation. Marine mammals are well adapted to demethylate, sequester, and eliminate mercury; data to quantify methylmercury exposure levels that would overwhelm these tolerance mechanisms remain elusive. Susceptibility of bats to methylmercury toxicity is a matter of increasing interest, and uncertainties in dose extrapolation are particularly significant for bats due to their small body sizes and high food ingestion rates.

TP034 More Than Just Point Estimates--Reintroduction to Probabilistic Methods for Toxicity Data

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A risk characterization that communicates key sources of uncertainty and variability associated with both exposure and toxicity offers risk managers a thorough understanding of the likelihood and magnitude of risks to ecological receptors. Following EPA's publication of "Policy for Use of Probabilistic Analysis in Risk Assessment at EPA" in 1997, numerous examples of probabilistic risk assessment (PRA) have been published. Particularly applicable to ecological risk assessment, probabilistic methods extend deterministic point estimates of risk (i.e., NOAEL- and LOAEL-based hazard quotients) through evaluation of distributions of exposure in the context of exposure-response relationships. This presentation focuses on probabilistic methods applied to the toxicity/effects assessment and reintroduces a method for developing composite exposure-response distributions when available data includes multiple studies, species, and effects endpoints. As an alternative to rank-ordered distributions of toxicity reference values (e.g., species-sensitivity distribution [SSD]), composite exposure-response distributions retain information on the slope of the exposure-response relationships and can accommodate study values for which the full exposure-response data set may be missing. We present a case study using fish toxicity data on polychlorinated biphenyls (PCBs) for a wide range of test species and effects endpoints. We explain how to apply the systematic approach and provide examples of visual displays of results and risk characterization conclusions that are supported by the available data.

TP035 Toxicity Reference Value Derivation: A Key Uncertainty that Influences Risk Estimates and Remedial Decisions

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A major challenge facing ecological risk managers is the integration of uncertainty in risk estimates into the risk management process. Uncertainty in risk estimates is often substantial, and failure to incorporate the degree of uncertainty in decision making processes can have a substantial effect on remedial costs and benefits. One key source of uncertainty in ecological risk assessments is the derivation of toxicity reference values (TRVs). TRV derivation can have a significant influence on the resulting risk estimate. Uncertainty related to the TRV is often propagated from risk estimates to remedial design and action through the development of risk-based preliminary remediation goals (PRGs). Examples are presented herein that demonstrate how uncertainties in TRV derivation translate into uncertainties in PRGs. These examples help elucidate ways to reduce uncertainty in TRV derivation by using approaches such as species sensitivity distributions (SSDs). These related topics are being addressed through the development of an American Society for Testing and Materials (ASTM) standard on risk evaluation at sediment sites, and the work related to TRVs and uncertainty in the ASTM document is presented herein.

TP036 Development of Tissue-Based Polychlorinated Biphenyl Toxicity Reference Values and Exposure-Response Curves for Fish Species

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Polychlorinated biphenyls (PCBs) are widespread pollutants that are known to bioaccumulate in aquatic ecosystems. Though surface water benchmarks can provide a preliminary assessment of PCB effects on fish, benchmarks of tissue concentrations represent a more direct and integrated measure of exposure and have stronger correlations with toxicological effects. We conducted a review of the primary literature on tissue-based PCB toxicity for freshwater and marine fish species. Effects related to reproduction, growth, and survival were selected as the basis for our toxicity assessment, because these endpoints are typically considered most relevant for assessing risks to ecological populations. Toxicity reference values (TRVs) were developed from published NOAELs and LOAELs as a threshold effects level (TEL) and a probable effects level (PEL). In addition, study data were used to fit exposure-response relationships, which can be used in probabilistic risk assessment to quantify effect levels and evaluate uncertainty in risk estimates.

Ecoxicoxicity of Per- and Polyfluoroalkyl Substances (PFASs)

TP037 Bioaccumulation, Bioconcentration, and Biotransformation of Emerging Poly-PFASs in Earthworms and Fish

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Per-fluoroalkyl substances (per-PFASs) are synthetic organic surfactants with a wide range of industrial and consumer applications. However, as is often the case with industrial chemicals, PFASs do not remain solely in their intended places. Once released to the natural environment they are not readily decomposable by physical and chemical mechanisms because of the strong carbon–fluorine bond. The widespread occurrence of PFASs in the environment coupled with their known adverse health effects on humans has aroused great concern for both the scientific community and the public. Recently, numerous poly-PFASs have been identified. In comparison with per-PFASs, our understanding of the removal, transport, and effects of these emerging poly-PFASs is very limited. This study focuses on the bioaccumulation/bioconcentration of a couple of poly-PFASs in earthworms and fish. The possible metabolism products of poly-PFASs were searched by a newly developed time-of-flight mass spectrometry approach based on continuously interleaving scans at low and high collision energies. The results will enhance our understanding the ecotoxicity of poly-PFASs.

TP038 Effects of individual perfluoroalkyl substances on Eisenia fetida in soil spiked at concentrations bracketing environmental relevance

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Perfluoroalkyls or perfluorochemicals (PFCs) which are persistent man-made substances have been used widely in various industries for almost 70 years. Among PFCs, long-chain perfluoroalkyl sulfonic acids (PFSAs) and perfluoroalkyl carboxylic acids (PFCAs) are of particular interest after scientists observed contamination of perfluorooctane sulfonic acid (PFOS) in wildlife and human blood contamination of perfluorooctanoic acid (PFOA). Perfluorohexanesulfonic acid (PFHxS), perfluorononanoic acid (PFNA) and perfluoroheptanoic acid (PFHpA) are long-chain PFCs included in the Toxic Substances Control Act while perfluorobutanesulfonic acid (PFBS) is a short chain PFC used as a replacement for PFOS. These compounds are frequently detected in food, human, and environmental samples along with PFOS and PFOA. Although some studies found PFBS, PFHxS, PFNA, and PFHpA to be less toxic than PFOS or PFOA, they are still of concern due to their persistence in the environment and potential to accumulate in terrestrial organisms. Earthworms are one of the organisms serving as a possible carrier of PFCs in contaminated soils to other organisms in foodwebs. The objective of our study was to evaluate the effects of PFBS, PFHxS, PFNA, and PFHpA to earthworms (*Eisenia fetida*) in soil contaminated with these compounds at 0.1, 1, 10, 1000, and 100,000 ng/g dry weight, covering concentration levels found in background, biosolid-amended, and facility-surrounding soils. Earthworms were exposed in PFC-spiked soil for 21 days. Results showed that there was no earthworm mortality after 21-d exposure in soils at concentrations lower than 1,000 ng/g. PFHxS, PFNA, and PFHpA caused the highest earthworm mortality (5%) which occurred only in 100,000 ng/g treatments, while PFNA caused lower mortality (2.5%) in both 1,000 ng/g and 100,000 ng/g treatments. Earthworm weight loss was at 4.4 - 29%; the highest weight loss was observed in worms exposed to PFNA at 100,000 ng/g. The concentration of target compounds in earthworms after 21-d exposure was ND – 127 mg/g wet weight. The ranking of PFCs concentrations in earthworms was: PFNA > PFHxS > PFHpA > PFBS. Our results are expected to fill some data gaps in PFC toxicity and provide helpful information relating to the potential for toxicity to higher organisms.

TP039 Exploring freshwater species sensitivity to environmentally persistent PFAS and PFAS mixtures

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Per – and Polyfluoroalkyl Substances (PFAS) are synthetic chemicals that are stable and persistent in the environment. PFASs were first discovered in 1938 by DuPont and trademarked as Teflon in the following years. In the 1960's the 3M company, in collaboration with the U.S. Navy developed the Aqueous Film Forming Foam (AFFF) to quickly and effectively extinguish fires. In 1999, EPA received information about the persistence, unexpected toxicity and bioaccumulation potential on PFOS. Subsequently, in 2000 3M announced it was discontinuing manufacturing perfluorooctanyl chemicals; despite the drastic domestic reduction of PFAS manufacturing, persistence of PFASs continues within all environmental compartments including, soil, water, air and in biota. Surface water near historically used fire training facilities and manufacturing plants have been reported to have PFAS contamination related to those activities. To date, most data available on the effects of PFAS to freshwater invertebrates were conducted as acute toxicity tests with the daphnid species, *Daphnia magna* exposed to Perfluoroalkyl sulfonic acid (PFOS) and Perfluoroalkyl carboxylic acid (PFOA). Out of thirty-six studies identified that tested PFAS on freshwater crustaceans, 24 used *D. magna* as the test species. Additionally, 19 of the 36 studies investigated the toxicity of PFOS and PFOA alone, 13 of which utilized *D. magna* as the test species. It should be noted however, that available studies indicate other crustacean species such as *Moina macrocopa* and *Chyrodus sphaericus* were the more sensitive to PFASs compared to *D. magna*. Additionally, within the dataset of sub-chronic exposures conducted with *M. macricopa* and *D. magna,* the species showed equal sensitivity to PFOS using a fecundity endpoint. Taken together, these data suggest *D. magna* may not be the optimal surrogate for freshwater crustacean sensitivity and future efforts will explore this hypothesis. Moreover, these studies investigated toxic effects of individual PFAS while PFAS in the environment is often present in mixtures, which may have interactions that elicit different physiological responses. Given the lack of available data on potentially more sensitive freshwater crustaceans and chronic exposures of environmentally persistent PFAS mixtures, which is the more likely exposure scenario, we have initiated zooplankton studies to explore and define the ecotoxicity of PFASs with limited data and as a mixture.

Challenges and Opportunities in Urban Agriculture – Nutrient Management and Contaminant Mitigation

TP040 Are urban gardens a source of P pollution?

G. Small, A. Kay, University of St. Thomas

Urban agriculture has expanded rapidly in recent years, and has potential to recycle nutrients from food waste into new food through the use of compost. However, in urban gardens, compost is often applied in excess of plants' phosphorus (P) demand, resulting in the buildup of P in the soil and potential loss through leachate or runoff. We measured P loss from experimental raised-bed garden plots that received one of two commonly used types of compost (municipal organics compost or manure compost) at three different application levels. Dissolved P concentrations in leaching from garden plots receiving manure compost was high, ranging from 5-11 mg P/L, depending on application level. Leachate P concentration from plots receiving municipal organics compost was an order of magnitude lower, ranging from 0.5-1.2 mg P/L, and leachate P in garden plots receiving no compost was 0.3 mg P/L. Total P lost through leachate during the growing season ranged from 0.4-1.4 g P/m^2 for manure compost treatments, compared to 0.04-0.24 P/m^2 for municipal organics compost treatments, and 0.02 P/m^2 for soil with no compost. Only one of compost treatments (high application rate of municipal organic compost) significantly increased crop yield relative to the control treatment. P lost from leachate and removed through harvest only represented 1-10% of total P applied as compost, suggesting that soil build-up was the dominant fate of P in this study. Our results indicate that urban gardens have the potential to act as point-sources of P pollution under certain management scenarios.

TP041 Ecological risk assessment of pesticide residues in fish samples from Donga River, Taraba State, Nigeria

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There is increasing need to monitor the potential risk of exposures to multiple residues of pesticides in our diets. Pesticide use in agricultural processes and irrigation farming along river banks have increased tremendously in recent times without considering the risk of exposure to aquatic biota which serves as good sources of food and nutrients in our diets. In this study, the levels of some pesticide residues commonly use in farming operations along river Donga, Taraba State, Nigeria were assessed from fish samples harvested from it. Five fish species popularly consumed in regular diet were collected for pesticide residues analysis using gas chromatography equipped with electron capture detector. The results showed that total pesticide residues (PR) were highest in *Synodontis membranaceus*, *Tilapia zill* and *Clarias gariepinus* at concentrations 0.09, 0.07 and 0.06 µg/kg respectively while the least total PR was obtained in *Heterotis niloticus* specie at 0.04µg/kg concentration. However, the mean distribution of PR were generally high for cyclodienes, DDT, DDD, dicofol methoxychlor aldrin, dieldrin and heptachlor depending on the species in the range of 0.006-0.009 µg/kg. Cyclodienes residues were the highest found in *Heterotis niloticus* at concentration of 0.013 µg/kg.

TP042 Fate of pharmaceuticals in human urine during reverse osmosis of source-separated urine from nutrient recovery

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Human urine source separation is a viable way to reduce nutrient loading on wastewater treatment plants and thus mitigate eutrophication of the aquatic environment in urban agriculture. Urine offers a natural source of nitrogen and phosphorous nutrients that are vital for plant growth. Commercial fertilizers undergo energy-intensive processes such as mining phosphorous and nitrogen fixation to provide plants with the required nutrients for growth. Currently, urine is treated in the wastewater system, and many plants have nutrient limitation requirements for effluent they must meet, to combat eutrophication of the aquatic environment which is responsible for hypoxic zones. Urine-derived fertilizers would mitigate eutrophication in wastewater by diverting the urine into collection bins where the urine can be concentrated and processed as a fertilizer. Urine also contains pharmaceuticals and hormones that are introduced into the wastewater, causing adverse effects on the organisms in the water. Using urine as a fertilizer feedstock could give the pharmaceuticals more time to degrade in the environment prior to introduction into the waterways, or could act as another pathway of environmental pharmaceutical exposure. The goal of this project is to assess the risks of urine-derived fertilizers by analyzing the uptake of pharmaceuticals and hormones in food crops. The urine-processing system in this study utilizes reverse osmosis and distillation to concentrate the nutrients in urine to be used as a fertilizer. Urine collected from the system is extracted by SPE, and pharmaceuticals and hormones are quantified by isotope dilution by LC-MS/MS and GC-MS/MS respectively. An extraction method for the distilled urine product is optimized to increase recoveries, decrease detection limits, and decrease matrix interferences. Fully concentrated urine from the system will be used to fertilize crops, and those crops will be analyzed for pharmaceutical and hormonal uptake. The risk of using urine as a fertilizer can ultimately be assessed by first better understanding the uptake of the pharmaceuticals and hormones found within the urine-fertilized crops.

TP043 Measuring the fate of P lost through leachate from urban gardens

S. Osborne, University of St. Thomas / Biology; G. Small, A. Kay, University of St. Thomas

Urban agriculture provides a variety of social, economic, and environmental benefits to communities, but also has the potential to act as a source of nutrient pollution. Although soils effectively bind phosphorus (P) at low soil P concentrations, above a soil P threshold of approximately 60 ppm, P losses increase linearly with increasing P concentration. Urban gardens have been shown to routinely have highly elevated soil P levels due to repeated compost application. P lost from urban garden leachate could eventually contaminate groundwater, but the fate of this excess P has been unexplored. We hypothesize that the soil P concentration and depth of penetration will increase over time after gardens are established. We conducted a survey of soil P concentrations at 11 urban farms in Minneapolis and Saint Paul, and additionally measured soil P concentrations along depth profiles below garden plots established between 2-7 years ago. Plant-available soil P concentrations across the 11 farms ranged from 139-341 ppm, more than an order of magnitude higher than recommended soil P levels. Following the demonstration of excess phosphorus, we began studying what happens to this phosphorus by studying garden plots established between 2-7 years ago. Soil samples taken from each plot at 4 different depths are analyzed for phosphate through 4 different extraction techniques to determine the amount of: 1) water-soluble inorganic and organic P, 2) weakly exchangeable inorganic and organic P, 3) Fe- and Al-bound inorganic P, and 4) organic fulvic- and humic-bound P. These results will allow us to better understand the fate of P lost via leachate from urban gardens, and specifically to quantify the rate of movement of this P towards the water table.

Integrated Understanding of Biogeochemical Cycling of Mercury Around Ocean Environments

TP044 Sources and cycling of methylmercury in coastal and offshore waters

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For humans and wildlife, exposure to methylmercury (MeHg), the most toxic and bioaccumulative form of mercury (Hg), is mostly from the consumption of seafood. Historically, coastal sediments have been viewed as the major source of the MeHg which is bioaccumulated by marine organisms, primarily because microbe-mediated production of MeHg has been demonstrated in marine sediments and other MeHg sources have been considered negligible. Overall, Hg speciation and the quality of organic matter have been pointed as major controls of net Hg methylation in coastal sediments. Contrary to this historical view, however, recent research highlights the presence of coastal marine ecosystems where sediments are not a significant source of MeHg to biota when compared to its other sources. For the open ocean, field measurements and Hg methylation/demethylation assays suggest that net methylation of Hg occurs in conjunction with organic matter degradation, mostly in lower oxygen subsurface waters. However, there is increasing evidence for the formation of MeHg in conjunction with the microbial loop of the surface ocean, where a substantial fraction of the organic matter produced via primary production is degraded. This presentation will explore the importance of Hg methylation in oxic surface waters as a source of MeHg to marine food webs. Estimates will be made based on mass balance calculations, which will include the results of recently conducted methylation/demethylation assays in seawater and sediments. The factors that influence the pathways of MeHg formation will be discussed, as will the processes of photochemically and biologically-mediated MeHg demethylation. Moreover, the detection of Hg methylating genes within bulk waters and in suspended marine particles will be highlighted as further evidence for microbe-mediated methylation of inorganic Hg in seawater, including the oxic water column. This recent research helps constrain the potential importance of Hg methylation in the water column, both in the open ocean and coastal waters, compared to other sources in providing MeHg to pelagic food webs.

TP045 Methylmercury production in the oceans: Links to physics, chemistry and biology

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We will review and synthesize the current understanding of the marine biogeochemical cycle of mercury and how its natural cycling may have been altered by anthropogenic emissions and changing climate. The presentation will focus on potential sources of methylmercury to the marine food web, which is of most socioeconomic and health concern. Marine methylmercury production is a function of at least three variables: inorganic mercury availability, organic matter supply and bacterial assemblages. All of these variables are prone to be influenced by global changes. We will make use of recently published and unpublished data to attempt evaluating the relative importance of these variables. With this we will examine the possible impacts of the temporal variation of anthropogenic mercury emissions and warming climate on marine methylmercury production. We will also point out remaining knowledge gaps, share ideas of future research needs and potential approaches to furthering our understanding of methylmercury dynamics in the ocean.

TP046 Mass budgets of methylmercury in Kongsfjorden, Svalbard

J. Kim, S. Han, Gwangju Institute of Science and Technology / School of Earth Sciences and Environmental Engineering

Methylmercury (MeHg) is a neurotoxin that bioaccumulates in aquatic food chains and human can be exposed to MeHg through fish consumption. While MeHg concentrations in Arctic seawater are relatively higher than lower latitudes, major sources of MeHg in arctic seawater remain uncertain. In the current study, we calculated mass budgets for MeHg in the Kongsfjorden, Svalbard, based on reference values and measurement data obtained from the 2016 and 2017 Svalbard cruise. We found out that significant MeHg production occurs in the water column through in-situ methylation, and input from sediment diffusion is the second largest source. The MeHg input from external sources, such as river and glacier, was relatively low. The result of mass flux estimation agreed well to the typical distribution pattern of MeHg in this region, showing higher concentrations (0.07-0.17 pM) at surface 0-50 m and lower at deeper depths (0.06-0.13 pM, 100-250 m). We also found two components of fluorescent dissolved organic matter from the surface seawater using a PARAFAC (parallel factor analysis) model: component 1 is biological organic matter like amino acid (i.e., tyrosine or tryptophan) and component 2 is terrestrial humic matter. The MeHg concentration was revealed to be higher in the C1-dominant seawater and lower in the C2-dominant seawater. Currently, we are measuring methylation (K_m) and demethylation rate constants (K_d) of Kongsfjorden seawater to find out how K_m and K_d are associated with environmental variables such as PARAFAC components.

TP047 Distribution characteristics of methylmercury and dissolved gaseous mercury in the Western Pacific Ocean

S. Han, Gwangju Institute of Science and Technology / Earth Sciences and Environmental Engineering; H. Kim, Korea Basic Science Institute / Seoul Center; T. Rhee, Korea Polar Research Institute

Methylmercury (MeHg) accumulation in marine organisms poses serious ecosystem and human health risk, yet the sources of MeHg in the surface ocean remain uncertain. We present the first mass budget estimation and distribution characteristics of MeHg and dissolved gaseous Hg (DGM) in the Western Pacific Ocean, based on the 2012 and 2014 SHIPPO survey (30°S-50°N). We found from the cruise data that the major net source of MeHg in surface water to be vertical diffusion from the subsurface layer (1.8 to 12 nmol m^{-2} yr^{-1}). A higher upward diffusion in the North Pacific (12 nmol m^{-2} yr^{-1}) than in the Equatorial Pacific (1.8–5.7 nmol m^{-2} yr^{-1}) agreed with elevated surface MeHg concentrations observed in the North Pacific (limit of detection-34 fM) as compared to the Equatorial Pacific (< limit of detection). On the contrary, surface water DGM concentration was found to be significantly higher in the Equatorial Pacific (100-300 fM) than the North Pacific (50-100 fM). The higher DGM in the Equatorial Pacific could be explained by redistribution of surface water in the tropical upwelling zone, supporting high primary production and dissolved organic carbon concentration. Our results suggest that MeHg and DGM distribution in surface of the Western Pacific Ocean is mainly controlled by the upward diffusion from subsurface water and the in situ reduction associated with the Ekman overturning process, respectively.

TP048 Redox transformation of mercury in coastal seawater: A case study in the Gwangyang Bay, Korea

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Mercury (Hg) in coastal seawater, transported from point and non-point sources, undergoes microbial and photochemical oxidation and reduction processes that are critical for fate and bioavailability of Hg. To understand how dissolved organic matter composition affects Hg redox transformation, we monitored total Hg, dissolved gaseous Hg (DGM), dissolved organic matter components, as well as typical water quality parameters in coastal seawaters. The surface seawater samplings for salinity, pH, chlorophyll-a, and Hg speciation have been performed at six to eight sites in the Gwangyang Bay located on the southwestern coast of Korea in 2015 and 2016. The Hg(II) and Hg(0) incubation experiments were also carried out to measure photoreduction (k_r) and photooxidation (k_o) rate constants of Hg. The results showed that concentrations of DGM, ranging from 12 to 690 fM, were higher at the higher chlorophyll-a sites, in general. The k_r and k_o ranged from 0.057 to 0.56 m^2 $MJ^{-1}$and from 0.18 to 0.40 m^2 MJ^{-1}, respectively, and there was a positive linear relationship between k_r and autochthonous dissolved organic component revealed by Excitation Emission Matrix-parallel factor analysis (EEM-PARAFAC). Overall, we demonstrated that the EEM PARAFAC is a useful tool to elucidate how dissolved organic matter composition affects Hg transformation processes in coastal seawater.

TP049 Mercury distribution in seawaters, planktons and fishes collected from the Kuroshio Current region of the East China Sea

K. Marumoto, National Institute for Minamata Disease / Environmental Chemistry Section; A. Takeuchi, National Institute for Environmental Studies / Center for Environmental Measurement and Analysis; N. Suzuki, National Institute for Environmental Studies / Center for Health and Environmental Risk Research

We measured the total Hg and methyl Hg in seawaters, planktons and fishes collected from the Kuroshio Current region of the East China Sea for evaluating Hg distribution and bioaccumulation in the sea area. This is because the East China Sea is the adjacent sea area of the Asian Continent which has been recognized as the largest Hg emission source in the world. The total Hg concentrations in the deeper waters were higher than those in surface and subsurface waters. On the other hand, the methyl Hg concentrations were also higher in deeper waters than in surface waters, and the maximum concentrations were observed in the depth of 500 – 600 m at the several sites. The temperature – salinity diagram shows that North Pacific Intermediate Water (NPIW) flows in this depth. The highest methyl-Hg concentrations in the NPIW were observed in the other sea areas such as the eastern Pacific Ocean and Equatorial regions. Therefore, it is possible that the horizontal advection of NPIW relates to high methyl Hg concentrations at the study area. From the results of the total Hg concentration in subsurface waters (~200 m) and planktons which were collected using a plankton net with 100 µm of mesh size, the bio concentration factors (BCFs) were calculated at 4.65 - 6.15. Although these values were almost equal with the reported values in other sea areas, they indicated that the total Hg in seawater was concentrated into the planktons from ten thousand to a few millions fold. In addition, we also measured nitrogen isotope ratios in the planktons and fishes and calculated the trophic magnification slopes (TMSs) of total Hg. The TMSs of total Hg in the Kuroshio Current region were slightly lower than those in other sea areas. Thus, the bio accumulation of total Hg between planktons and fishes were not remarkable in the study area. The BCFs and TMSs of methyl Hg will be also calculated and discussed in our presentation. This research was supported by the Environment Research and Technology Development Fund (5-1405) and (5-1702) of the Ministry of the Environment, Japan.

TP050 Comprehensive assessment for controlling factor of total Hg level in skipjack tuna from Western North Pacific Ocean

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Fish represent the primary source of mercury (Hg) to human populations. The predominant Hg form for the human intake is methylmercury (MeHg), since this form is highly bioaccumuulative in aquatic environment. Despite low level of MeHg in natural water, bioconcentration to microseston magnify the concentration in 3-5 orders, then food chain transfer contribute to further increase of MeHg in higher pradators. Migratory pelagic marine species such as tuna are particularly significant source of MeHg to human. It is important to establish the model which can predict possible change in MeHg in pelagic fishes considering change in Hg input to ocean or global climate. However, current understanding are not sufficient to explain highly variable MeHg level among individuals, populations, and species due to confounding of biogeochemical, ecological, and biochemical factors. Even among the same species, considerable geographical variation have been reported. In this study, we measured total Hg (THg) level in muscle of 251 skipjack tuna collected from Western North Pacific Ocean covering Kuroshio-Oyashio transition zone (KOTR, n=50), Kuroshio zone (KR, n=122), Japan Sea (JS, n=20), East China Sea (ECS, n=51), and North Equator Counter Current zone (NECC, n=13). The year of sampling were ranged from 1997 to 2003, and all samples were cryogenically archived to environmental specimen bank, Ehime University. Average THg level in muscle (fresh weight) were 0.24±0.21 (KOTR, n=), 0.30±0.25 (KR), 0.35±0.14 (JS), 0.32±0.27 (ECS), 0.08±0.02 µg/g (NECC). There are some geographical variation, while inter-regional variation was also large. Although the predominant factor affecting inter-region variation was body size, other parameters were also significant. In the presentation, we will provide comprehensive data analysis for the controlling factor of THg level in this species considering physiological parameters, trophic position, regional variation, temporal/seasonal variations, and mercury stable isotope signature.

TP051 Mercury (Hg) isotopic variations of fishes from coastal, marginal, and pelagic marine ecosystems within exclusive economic zone (EEZ) of Japan

A. Takeuchi, Y. Hanamachi, National Institute for Environmental Studies; K. Marumoto, National Institute for Minamata Disease / Environmental Chemistry Section; K. Mori, National Institute for Minamata Disease; A. Noda, Tokyo University of Marine Science and Technology; Y. Shibata, National Institute for Environmental Studies; N. Suzuki, National Institute for Environmental Studies / Center for Health and Environmental Risk Research

Mercury (Hg), a potent harmful trace metal, is a global pollutant and present in all environmental compartment. A large amount of Hg presents in the global ocean, and methylated form of mercury (MeHg), a highly toxic compound, can be bioaccumulated and biomagnified in marine food chains. Fishes are important protein sources in human and wildlife diets worldwide, and consumption of them can pose human and ecological health risks. In this study, Hg isotopic compositions of various fishes within exclusive economic zone of Japan were measured to distinguish different biogeochemical processes and sources of bioaccumulating Hg in different marine environmental settings. Fish samples were from a coastal ecosystem of Minamata Bay, a marginal marine ecosystem from Genkai sea, and pelagic ecosystem in Northwest Pacific Ocean. The determined d^{202}Hg and D^{199}Hg of pelagic fishes (d^{202}Hg: 0.4 – 1.0‰, D^{199}Hg: 1.6 – 3.0‰) indicate their relatively higher isotopic ratios than those of fishes from marginal sea (d^{202}Hg: 0.2 – 1.25‰, D^{199}Hg: 0.8 – 1.5‰) and coastal environment (d^{202}Hg: -0.7 – 0.4‰, D^{199}Hg: 0.1 – 0.7‰). The determined d^{202}Hg variations can be explained by biotic and abiotic demethylating processes, and the D^{199}Hg variations can be attribute to different magnitude of photo-reduction. Also, the D^{199}Hg variations are related to water depth of their sampling locations. Water depth of the pelagic environment exceeds 1,000 m, and the water depth of the marginal marine ecosystem ranges from 100 to 200 m. The D^{199}Hg values of pelagic fishes were approximately 0.1 to 3.5‰ higher than those from marginal marine ecosystem. The D^{199}Hg values of fishes from Minamata Bay were the lowest where its water depth is less than 30 m. It has been known that sediment is a major component where MeHg is produced, and the determined d^{202}Hg and D^{199}Hg values of coastal and pelagic sediments ranged from -1.3 to -0.5‰ in d^{202}Hg and 0 to 0.2‰ in D^{199}Hg. The Hg isotope compositions of coastal fishes were nearly identical to the sediment Hg isotopic values. Hg isotope compositions of fishes are useful proxies to investigate the aquatic Hg biogeochemical and bioaccumulating processes. Also, the fish Hg isotope compositions can be used to indicate different habitat related to their water depth and characterize exposure route of MeHg to human and wildlife.

TP052 Mercury sources and budgets in the upper ocean: Results from the global multimedia model FATE-Hg

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To evaluate the effectiveness of international efforts to reduce anthropogenic emissions, it is important to understand the changes in mercury concentrations in a region that result from emission reductions in a foreign source region. A new global multimedia model, the Finely-Advanced Transboundary Environmental model for mercury (FATE-Hg), was developed and applied to estimate mercury sources and budgets in the global oceans. The principal characteristic of this model is that it is based on a coupled atmosphere-ocean transport model, and calculates 3D non-steady physical transport in both the atmosphere and the ocean. Additionally, it considers methylated mercury production in the water column followed by biotransfer from lower (i.e., particle organic matter) to higher (i.e., fish) order marine consumers. We defined eight continental source regions and 19 ocean receptor areas (FAO major fishing areas), and estimated the source-receptor (S-R) relationships using the emission sensitivity method. From the results of S-R analyses, East Asia was the dominant source region in the Northern Hemisphere, with the exception of the Mediterranean and with a maximum contribution of 55% in the Northwest Pacific. Conversely, in the Southern Hemisphere, contributions from Australia and Oceania, South America, and Africa generally dominated. Sources of mercury in higher order marine consumers (e.g., tuna, bonito, and billfish) caught in several different countries were estimated using the results of S-R analyses and FAO global fishery statistical data. The results provide useful information on how to reduce mercury exposure in people in the countries analyzed.

The Ecotoxicology of Elevated Major Ion Mixtures in Fresh Waters

TP053 Acute and chronic toxicity of sodium nitrate to three freshwater organisms in water-only exposures

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To facilitate the development of US national ambient water quality criteria and State water quality standards for nitrate, studies on the toxicity of nitrate to a diverse suite of aquatic organisms are needed. The objective of this study was to evaluate acute and chronic toxicity of nitrate (as sodium nitrate) to a unionid mussel (fatmucket, *Lampsilis siliquoidea*), a midge (*Chironomus dilutus*) and rainbow trout (*Oncorhynchus mykiss*). The test species were selected based on the need for meeting the US Environmental Agency's guideline minimum data requirements for developing water quality criteria. Acute toxicity tests (48 hours with midges and 96 hours with mussels and trout) and chronic toxicity tests (28 days with mussels, 10 days with midges, and 40 days with trout) were conducted in diluted well water (hardness of 100 mg/L as $CaCO_3$) following ASTM International standard methods. Among the three species, the midge was more sensitive to nitrate than the mussel and trout in both acute and chronic exposures, and the mussel was more sensitive than the trout in chronic exposures. When the acute EC50s and chronic EC20s were included in the compiled nitrate toxicity database for all freshwater species, the midge and mussel were among the four most sensitive species for chronic exposures.

TP054 Comparative analysis of toxicity of simulated mine effluents on native Ephemeroptera taxa

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Standard toxicity testing organisms and endpoints are utilized for regulatory purposes and often ecological risk assessments. However, these organisms and endpoints may not be the most sensitive and may not reflect responses of all native taxa. Based on field surveys, mayfly taxa have been reported to be more sensitive to aquatic contaminants than the standard toxicity test organisms used in determining effects on aquatic ecosystems. Laboratory testing of these sensitive taxa has been hindered by the inability to culture the organisms in the laboratory. Further, it is essential that toxicity testing be conducted with the most sensitive life stage, usually the juvenile stage. Eggs were collected from native mayflies via oviposition or dissection and both eggs and nymphs were exposed to a simulated mine effluent representing exposure to elevated conductivity in mining influenced Appalachian streams. Endpoints evaluated were hatch rate, incubation period, survival and growth of juveniles exposed to the elevated conductivity pre- and post-hatch. Multiple taxa were evaluated with Ephemeridae (*Hexagenia sp.*), Heptageniidae (*Epeorus sp.*) and Baetidae (*Baetis sp.*) reported herein. Updates are also given on rearing methods of the multiple taxa.

TP055 ECp estimation methodology for the chronic toxicity of NaCl to Ceriodaphnia dubia

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For chronic toxicity to aquatic organisms, the past 30 years have seen a growing interest in the estimation of concentrations causing a certain percentage effect (ECp) rather than using highest no-observed effect concentrations (HNOEC) or lowest observed effect concentrations (LOEC) as the basis for risk characterizations. Although ECp estimation has certain advantages for defining risk, it intoduces various questions regarding model selection, parameter estimation, and data sufficiency. A large test of the chronic toxicity of NaCl to *Ceriodaphnia dubia* was conducted using the standard protocol of 1 initial organism per test chamber and a 7-day duration, but consisting of 16 concentration levels, each with 6 – 30 replicate chambers, for a total of 360 experimental units. Survival of the initial test organisms and production of offspring were used to assess the shape of the exposure/effects curve and other statistical properties, and to develop an ECp methodology appropriate to these data, including integrating mortality with the reproduction of surviving organisms to assess total impact on reproduction. Issues with adapting such methodology to more typical datasets were assessed. The contents of this abstract neither constitute nor necessarily reflect USEPA policy.

TP056 Effects of elevated major ion concentrations on energy storage in freshwater mussels: Comparison of laboratory and field studies

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Coal mining activities have caused total dissolved solids concentrations to triple in the upper Powell River (Virginia, USA) since monitoring began in the 1940s. At present, measured specific conductivity is frequently greater than 800 µS/cm at baseflow. The Powell River also supports a diverse assemblage of freshwater mussels, but species richness has declined dramatically in the upper river. We conducted a laboratory study to evaluate the effects of elevated concentrations of major ions and coal-contaminated sediment on energy storage in adult freshwater mussels, and compared the results to those of an observational field study. For the laboratory study, mussels (*Lampsilis fasciola*) were exposed to pond water, simulated Powell River water, clean sediment, and Powell River sediment in a 2 x 2 full factorial design for six weeks. There was no mortality in any treatment. Energy storage, measured as glycogen in mantle tissue, was significantly different between male and female mussels ($p=0.0092$). There was a significant effect of water treatment on glycogen content for male mussels only ($p=0.014$), with lower glycogen content in males exposed to simulated Powell River water. There was a significant effect of water treatment on mussel condition index ($p<0.015$), measured with several different methods. Mussels exposed to simulated Powell River water had lower body condition, regardless of gender. We hypothesize that exposure to elevated major ion concentrations caused mussels to utilize energy stores to compensate for osmotic stress, with males utilizing glycogen and females utilizing lipids from oocyte resorption. These results are comparable to the observational field study, where *L. fasciola* were held in silos in three rivers affected by coal mining activities for eight weeks. There was no mortality of any individuals, but mussels held in the Powell River (mean specific conductivity = 850 µs/cm) had lower glycogen content and condition index than mussels held in the other two tributaries (mean specific conductivity = 470 and 676 µs/cm). Taken together, these results suggest that elevated major ion concentrations affect energy storage in individuals without necessarily affecting survival, which has important potential implications for reproduction and long-term impacts on populations.

TP057 How statistical artifacts affect the reliability of toxicity thresholds calculated from distribution tails of field monitoring data

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Historically, toxicity-based thresholds considered protective of aquatic life were developed from laboratory experiments. These experiments have the advantage of controlling exposure to different levels of the stressor of concern while holding constant exposure to other stressors, isolating effect and inferring causality directly and confidently. More recently, statistical methods have been proposed for inferring single-stressor causality from uncontrolled field biomonitoring data. These methods assume taxa presence data represent tolerance to a particular stressor and select a criterion value from the left tail of a field-based "taxa sensitivity distribution," which is deemed protective of the majority of taxa. However, without controlling for the uneven sampling effort across taxa and confounding effects of other stressors, the application of such methods may inaccurately quantify the toxicological effect of the stressor of concern and result in an unreliable environmental criterion. We examine several datasets provided as part of the U.S. Environmental Protection Agency's (EPA's) draft *Field-Based Methods for Developing Aquatic Life Criteria for Specific Conductivity* to evaluate the appropriateness of using the tail of the field distribution of taxa sensitivity to conductivity for setting a criterion for conductivity in water. EPA recommends using environmental biomonitoring data that sample biological communities in the field along with co-occurring physical and chemical water quality parameters. These samples characterize a "snapshot" condition of a stream and are not a representation of all taxa at a sampling location. As such, individual taxa are sampled disproportionately in this kind of data collection effort, because some taxa may be present in a sample much less often than other taxa due to rarer population abundance or simply random chance. As sample size affects the accuracy of summary statistics, such as a 95th percentile extirpation concentration, differences in sample sizes among taxa can create statistical artifacts misinterpreted as meaningful relationships between stressor levels and biological community response. Field monitoring data also do not control for varying values of all the stressors that cumulatively affect benthic invertebrates, which can cause misattribution of effects among stressors varying concurrently. Such statistical artifacts undermine confidence in derived criteria values intended for regulatory use.

TP058 Influence of multi-ion exposures on Na+/K+-ATPase and Carbonic Anhydrase activity in Pimephales promelas (Fathead Minnow)

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Dissolved ions have several important physiological functions within aquatic organisms. Primarily, ions create electrochemical gradients that ultimately control the movement of water and other ions between the internal and external environment of these organisms. In freshwater species, the internal ion concentration is typically greater than that of their external environment. As a result, freshwater fish must utilize active transport through a series of pumps and transporters in order to maintain a homeostatic balance. However, many anthropogenic activities, including agricultural irrigation, road-salt runoff, and mining operations produce elevated ion concentrations within freshwater systems. An increase in dissolved ions directly corresponds to an increase in salinity. If the external ion concentration exceeds what is physiologically tolerable for freshwater organisms, more energy will be allocated towards ionoregulation, resulting in a decrease in energy available for other important functions, such as reproduction and growth. In order to better understand this change in energy allocation as a result of exposure to anthropogenically increased ion mixtures, fourteen-day static, renewal exposures were conducted using adult fathead minnows (*Pimephales promelas*). Fish were exposed for seven days to the sodium salts of chloride, sulfate and bicarbonate as single ions and in binary mixtures to determine the response of energy dependent enzymes involved in ion regulation: carbonic anhydrase and sodium-potassium ATPase. A seven-day recovery period was allowed in order to elucidate how quickly these freshwater organisms return to normal enzymatic functions. The results of this work provides further insight into the chronic toxicity of ion mixtures to freshwater organisms, and serves as a foundation for the development of predictive models for managing water quality.

TP059 Investigating the Sublethal Effects of Coal Ash Contaminants on Fathead Minnows (Pimephales promelas)

S. Edmonds, L.T. Yonkos, University of Maryland / Environmental Science and Technology

The project investigates the toxicological consequences of leachate from "dry" coal ash disposal on water quality and biota in receiving waters. The on-going study includes laboratory-based toxicity testing of field-collected media (e.g., impacted sediment and surface water) and simulated leachate generated from "fresh" coal combustion residues. Priority metals and other contaminants have been identified and monitored within ash field leachates and along gradients from three Maryland disposal facilities. A series of whole effluent toxicity tests and sediment toxicity tests have been completed using surface water and sediment samples from receiving streams in proximity to these facilities. Results indicate multiple regions of significant acute and chronic toxicity. Contaminated landscapes have been delineated and regions of potential toxicological impact have been determined based on threshold exceedances. Lethal and sublethal exposure thresholds of laboratory generated leachates have been determined in 7-day static renewal bioassays with common laboratory species (e.g., fathead minnow *Pimephales promelas*; *Ceriodaphnia dubia*). Investigation of behavioral toxicity associated with sub-lethal leachate exposures to larval and juvenile fathead minnows is on-going. Behavioral analyses will determine the potential for lasting impacts in juvenile fish following early life stage exposure compared with fish exposed later in life. Behavior responses being quantified include impaired swimming performance and delayed response to stimuli. Morphological and physiological changes resulting from exposure to coal ash leachates have the potential to critically inhibit behaviors associated with prey acquisition, predator avoidance and mating success. Results of behavioral analyses will be presented and population and community-level impacts will be discussed.

TP060 Laboratory investigation on the effects of conductivity on a sensitive early lifestages of fish from the Appalachian region

L. Beach, K. Tasker, Marshall University; M.Y. Armstead, Marshall University / Natural Resources and Environmental Science; M. Wilson, Marshall University / IST

While it is known there is a link between land disturbance and elevations in ionic constituents in streams, the causal relationship between elevated conductivity and aquatic taxa impairment is harder to define. Multiple field studies demonstrating correlations between conductivity and fish or benthic macroinvertebrate communities have not described the mechanisms of impairment and impairment has not been demonstrated with traditional toxicity testing. In an effort to explore more sensitive sub-lethal endpoints for evaluation of instream effects of mining effluent, chronic toxicity testing was conducted on eggs and early life stages of trout species utilizing a simulated mining discharge with elevated conductivity. Chronic toxicity testing conducted with the native taxa and sub-lethal endpoints were utilized to evaluate the relationship between conductivity and organism fitness without the variability associated with field studies. Embryo-larval and standard chronic larval toxicity testing was conducted on sensitive life stages of brook trout (Salvelinus fontinalis) and rainbow trout (*Oncorhynchus mykiss)* using a high sulfate synthetic mine effluent. Testing was also conducted using the standard test organism, fathead minnows (*Pimephales promelas)*. Comparison of response between taxa and between life stages of individual taxa's were made. Conductivities ranged from 100-2400 µS/cm in the exposures with mortality and teratogenesis being the endpoints in embryo-larval testing. Embryo exposures were initiated at fertilization in the fathead minnow tests and at 3 days old for rainbow trout, with both having EC50s greater than 2430 µS/cm. Generally, there was little sensitivity in the embryo or larval exposures with endpoints consistently >2430 µS/cm. Estimated effect concentrations (IC20s) were variable between the species and the life stages indicating that not only are the tolerance levels of each species different, but the tolerance of the life stages of each species is also variable.

TP061 Species-Specific Toxicity of Major Ion Salts 1: Fathead Minnows and Pond Snails

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Elevated major ion concentrations (Na, K, Ca, Mg, Cl, SO_4, HCO_3) have been recognized as a cause of surface water impairment and the toxicity of these major ions has been shown to be dependent on the specific ion composition of the water. A long-term research project was initiated at the USEPA Mid-Continent Ecology Division-Duluth to develop models that predict the toxicity of combinations of major ions. Research efforts initially focused on characterizing the toxicity of major ions and salt mixtures to *Ceriodaphnia dubia*. Subsequently, tests have been conducted with additional species with the goal of expanding the models to be more inclusive so that they can be better used to estimate risk in aquatic environments. This poster presents the results of acute toxicity tests conducted with two additional species: the fathead minnow (*Pimephales promelas*) and the great pond snail (*Lymnaea stagnalis*). Over 200 acute toxicity tests were conducted with these species to determine: 1) the effect of the ion composition of background (dilution) water on the toxicity of salts and 2) the toxicity of different salt mixtures. Generally speaking, fathead minnows and snails are less sensitive to major ion salts than are *C. dubia*. There are also some notable qualitative differences in their response to mixtures; for example, mixtures of Na and Mg salts indicate independent action of two mechanisms for *C. dubia*, but concentration additivity and a single mechanism for fathead minnows. Such differences suggest differences in toxic mechanism among the species. The contents of this abstract neither constitute nor necessarily reflect USEPA policy.

TP062 The Identification and Confirmation of Ionic Imbalance as an Effluent Toxicant for a Drinking Water Production Facility

M.K. Chanov, EA Engineering, Science and Technology / Ecotoxicology; W.L. McCulloch, EA Engineering, Science, and Technology, Inc., PBC / Ecotoxicology; M.D. Jirsa, M. Durbano, J. Biondo, EA Engineering Science and Technology Inc PBC

The increased efficacy of wastewater treatment has resulted in the removal of the majority of classical toxicants in industrial wastewaters. However, as a result, the concentrations of total dissolved solids (TDS) in industrial wastewaters has become increasingly important. Previous failing NPDES whole effluent toxicity testing events at a drinking water production facility prompted a toxicological evaluation of their final effluent. In order to evaluate the toxicity of the facility's effluent, EA conducted chronic whole effluent toxicity (WET) testing with the opossum shrimp, *Americamysis bahia* and the sheepshead minnow, *Cyprinodon variegatus* as the test species. The WET testing indicated that the samples showed toxicity to *A. bahia,* but not to *C. variegatus.* The samples were further evaluated utilizing combinations of standardized and innovative toxicity identification evaluation (TIE) procedures to identify the dominant toxicants in the complex wastewater using *A. bahia* as the only test species. USEPA's Phase I TIE methods were used to characterize the physical and chemical nature of the toxicants. Phase II TIE methods were used to further characterize and identify the cause(s) of toxicity in the effluent, and Phase III methods were used to help confirm that the suspected toxicants are indeed the cause(s) of the measured toxicity. Following completion of Phase I and II evaluations, the results indicated that an imbalance of dissolved ions was the primary toxicant in the effluent causing unsatisfactory whole effluent toxicity performance for *A. bahia*. Therefore, EA conducted a Phase III TIE evaluation, utilizing mock effluent studies and analytical verification of ion concentrations. Mock effluents were prepared using the results of the ion scans input into the Gas Research Institute (GRI) Salinity Toxicity Relationship (STR) Model. Following, completion of the Phase III mock effluent testing, the results of the mock effluent were compared to the real effluent sample tested concurrently. The results of the Phase I, II and III TIE testing identified and confirmed ionic imbalance as the primary toxicant associated with the toxicity observed to *A. bahia*. Following completion of the study, the permittee was able to successfully renegotiate the NPDES permit requirement for the facility to exclude *A. bahia* as a test species based on the findings of the TIE.

TP063 The Identification of Total Dissolved Solids as the Primary Effluent Toxicant for an Industrial Manufacturing Facility

M.D. Jirsa, EA Engineering, Science, and Technology, Inc., PBC; M.K. Chanov, EA Engineering, Science and Technology / Ecotoxicology; W.L. McCulloch, EA Engineering, Science, and Technology, Inc., PBC / Ecotoxicology; J. Biondo, EA Engineering Science and Technology Inc PBC

The increased efficacy of wastewater treatment has resulted in the removal of the majority of classical toxicants in industrial wastewaters. However, as a result, the concentrations of total dissolved solids (TDS) in industrial wastewaters has become increasingly important. Previous failing NPDES whole effluent toxicity testing events at an industrial manufacturing facility prompted a toxicological evaluation of their final effluent. Chronic whole effluent toxicity testing was conducted, incorporating a multi-species approach using *Ceriodaphnia dubia, Daphnia magna,* and *Pimephales promelas.* The multi-species testing indicated that the total osmotic strength of the effluent may be causing the toxicity and not a single chemical contaminant. The chronic toxicity testing showed that *C. dubia* was the only species to exhibit a toxicological response, as the samples were non-toxic to *D. magna* and *P. promelas*. The comparison of the toxicity testing results indicated that only the more TDS sensitive water flea had reproductive impairment when exposed to the effluent. Therefore, a Phase I chronic toxicity identification evaluation (TIE) was conducted using *C. dubia* as the test species. Concurrently, a review of previous toxicity test results and analytical data collected for the effluent was conducted. The review indicated that TDS may be a major contributor to toxicity as the concentrations of chloride, sulfate and bicarbonate were above published individual ion concentrations expected to cause toxicity. Furthermore, the results of the TIE showed that none of the manipulations reduced the toxicity of the sample, supporting TDS as the primary toxicant in the wastewater. Combining the results of the TIE, the multi-species testing, previous toxicity test results, and analytical data it was concluded that total dissolved solids were the primary toxicant associated with the effluent from this facility.

TP064 Species-Specific Toxicity of Major Ion Salts 2: Hyalella azteca, Lumbriculus variegatus, and Chironomus tentans

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Elevated major ion concentrations (Na, K, Ca, Mg, Cl, SO_4, HCO_3) have been recognized as a cause of surface water impairment and the toxicity of these major ions has been shown to be dependent on the specific ion composition of the water. A long-term research project was initiated at the USEPA Mid-Continent Ecology Division-Duluth to develop models that predict the toxicity of combinations of major ions. Research efforts initially focused on characterizing the toxicity of major ions and salt mixtures to *Ceriodaphnia dubia*. Subsequently, tests have been conducted with additional species with the goal of expanding the models to be more inclusive so that they can be better used to estimate risk in aquatic environments. This poster presents the results of acute toxicity tests conducted with three additional species: amphipods (*Hyalella azteca*), oligochaetes (*Lumbriculus variegatus*) and midges (*Chironomus tentans*). Over 200 acute toxicity tests were conducted with these species to determine: 1) the effect of the ion composition of background (dilution) water on the toxicity of salts and 2) the toxicity of different salt mixtures. Midges and *L. variegatus* are less sensitive to major ion salts than are *C. dubia*. Amphipods are more sensitive to some salts (e.g., $MgSO_4$ and Na_2SO_4), but less sensitive than *C. dubia* to others (e.g., NaCl and $CaCl_2$). There are also some notable qualitative differences in their response to mixtures. For example, while osmolarity is a good predictor of toxicity to *C. dubia*, Na_2SO_4 is more toxic to amphipods than can be accounted for by osmolarity. Differences in response suggest differences in toxic mechanism among the species. The contents of this abstract neither constitute nor necessarily reflect USEPA policy.

Fate and Effects of Metals in Organisms – Biogeochemical and Subcellular Perspective

TP065 Binding of trace elements to cytosolic biomolecules in metal-exposed aquatic animals (insect, fish)

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Subcellular partitioning measurements have been used to evaluate trace element toxicity and detoxification in several aquatic organisms. However, this approach does not reveal detailed information about the different biomolecules involved in binding such contaminants. In this context, previous subcellular metal partitioning studies in two groups of aquatic animals (insect: *Chaoborus*; fish: *Perca flavescens* and *Anguilla rostrata*) showed that substantial intracellular Tl and Ni burdens were found in the heat-stable protein (HSP) fractions (which includes metallothioneins, MT, and metallothionein-like proteins, MTLP), but the molecular entities targeted remained unknown. To characterize these biomolecules, a hyphenated technique was applied to the HSP fractions collected using whole animals (insect) or hepatic tissue (fish) from field-collected organisms. Size-exclusion chromatography coupled to inductively-coupled plasma mass spectrometry (SEC-ICP-MS) was used to separate and characterize cytosolic, thermostable metal complexes. Subsequently, the complexes containing Tl or Ni were collected from the SEC system and further analyzed using molecular mass spectrometry. Our results showed that neither Tl nor Ni was associated with metallothioneins or metallothionein-like proteins, which reflects the preferential binding of these metals to functional groups other than the MT thiols. Thallium consistently targeted a low molecular weight ligand (LMW, insect: < 1.3 kDa; fish < 1.1 kDa), which responded to increasing Tl concentrations in the HSP fraction by increasing its concentration. With regards to Ni, distinct ligands varying in their molecular weight (MW) were bound to this contaminant, but with a higher MW than that observed for Tl. Candidates for the biomolecules involved in binding Ni and Tl in the cytosolic compartment of each organism studied will be discussed. These results provide important insights into the intracellular trafficking of metals in aquatic organisms chronically exposed to non-essential metals, which contributes to our understanding of metal toxicity.

TP066 Characterisation of Ni-Biotic Ligand Interactions in Seawater

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The binding of metal by ligands is assumed to reduce the bioavailability of metal to the biotic ligand (organism), thereby reducing toxicity in the system and this relationship can be predicted using the biotic ligand model (BLM). However, the effects of nickel (Ni) in saltwater ecosystems has not received as much research attention as freshwater (FW) ecosystems. Thus, there is a pressing need to obtainexperimental data and to test models of Ni binding in order to predict, mitigate, and manage potential Ni pollution and toxic impacts. My study predicts that model ligands will reduce short term toxicity (EC_{50}) to purple sea urchins (*S. purpuratus*) and that this toxicity will vary depending on the binding affinity ($logK_f$) of the ligand. It is hypothesized that the EC_{50} of metal free ion (Ni^{2+}) will be similar between model ligands if toxicity is explained by [Ni^{2+}]. It is also hypothesized that the binding affinity of the biotic ligand can be determined using known binding affinity constants. Defined solutions of artificial seawater (ASW) containing different model ligands (i.e. ethylenediaminetetraacetic acid (EDTA), glutamic acid (GA), tryptophan (TRY), histidine (HD), and citric acid (CA)) were used for Ni toxicity and speciation tests. Total dissolved Ni concentration [Ni_D] of the treatments has been measured using the Graphite Furnace Atomic Absorption Spectrometer (GFAAS) and [Ni^{2+}] has been predicted using Visual Minteq 3.1. The dose-response curves were both expressed as % abnormal development of the sea urchin embryo by [Ni_D] and [Ni^{2+}] and corresponding EC_{50} values were determined. The methods developed with model compounds can be applied to natural samples in order to determine the binding affinity and protectivity of dissolved organic matter (DOM) and Ni EC_{50} values. The results showed that: 1. protection of the biotic ligand occurred when model ligands were added; 2. protection increased with increased Ni binding affinity; 3. consistent with the BLM, Ni toxicity can be explained by [Ni^{2+}]. The results of this research contribute to the development of biotic ligand-based prediction models for estimating the impacts of Ni in marine water. Funding was provided by Natural Sciences and Engineering Research Council of Canada (NSERC), VALE and NiPERA (Nickel Producers Environmental Research Association).

TP067 Deciphering the Aquatic Toxicity of Metal Mixtures from Breccia Pipe Uranium Mine Ponds

J.A. Steevens, D. Cleveland, C.A. Richter, J.K. Kunz, J. Hinck, USGS / Columbia Environmental Research Center

The Grand Canyon watershed in northern Arizona, USA contains relatively high-grade uranium deposits associated with breccia pipe formations. The USGS and its partners are currently studying active mine sites, as well as post, and pre-mining sites in order to inform on the ecological effects of breccia pipe uranium mining. The individual mines are composed primarily of the mining shaft, shaft overburden, ore pile, and a retention pond designed to contain water from the mine shaft and runoff from the site. Previous data that examined the leaching of metals from ore and waste rock indicate arsenic, cobalt, molybdenum, nickel, and uranium may leach from the overburden rock that is generated during the sinking of the shaft and from ore stored at the mine surface. One management concern is that water in the retention pond may act as an attractive nuisance for biota in this arid region, and the biota may be exposed to these metals. To assess the potential exposure and effects of metals from the site, sediment and water samples were collected from the mining site to characterize the water quality properties, metal chemistry, and biological effects. Sediment toxicity experiments were conducted using a 28-day *Hyalella azteca* bioassay. Toxicity of water samples was determined using *Ceriodaphnia dubia*. In addition to the field collected samples, laboratory generated mixtures were used to determine the aquatic toxicity of metal mixtures using the *C. dubia*. Chemistry and toxicity data from this site, as well as laboratory toxicity mixture studies, are being used to determine if the toxicity of the metal mixtures can be accurately predicted using mixture models based on individual metals. Ultimately these data will support the stewardship of lands associated with breccia pipe uranium mining in the Southwest United States.

TP068 Effect of metal mixtures on uptake by marine phytoplankton (Thalassiosira weissflogii): Copper, Nickel, and Cadmium

H. Kim, S. Jeong, Korea Basic Science Institute / Seoul Center; K. Chung, Korea Institute of Geoscience and Mineral Resources; H. Yoon, Korea Basic Science Institute / Seoul Center

Water quality criteria are generally based on results of toxicity tests with single compounds only. To date, several researchers have suggested that joint effect of toxic compounds should be taken into account for better approach. Attempt to model the joint effect have already been made for the fresh water environment, but the information on the marine environment is still limited. The effect of metal mixtures such as copper(Cu), nickel(Ni), and cadmium(Cd) on the marine environment were examined using marine phytoplankton(*Thalassiosira weissflogii*). Cells were exposed to the metal mixture(Cu-Ni-Cd) under different mixture composition and concentrations. Here, the concentrations of the individual metals of the mixtures were calculated using the concentration-addition model. The metal uptake rates and intracellular hydrolysis rates of fluosescein diacetate (FDA), used as a probe for metal viability under metal stress, were determined from metal exposed seawater. The single-metals showed relatively simple kinetics with an increase in intracellular uptake and metal stress as a function of exposure time. However the metal mixtures(Cu-Ni-Cd) competed for intracellular uptake through cell membrane. These results are important in understanding the accumulation of metal mixture in response to metal-metal interactions in seawater.

TP069 Trace element concentrations in surface waters and their relationship to fish otolith chemistry: Evidence of signatures from hydroelectric impoundment

A. Vanderpont, University of Manitoba / Environment and Geography; N.M. Halden, University of Manitoba / Geological Sciences; M.L. Hanson, University of Manitoba / Environment and Geography

In Manitoba, Canada the Coordinated Aquatic Monitoring Program (CAMP) is a long-term, system-wide water management program for much of province's hydroelectric impounded and non-impounded sub-basins. CAMP monitoring includes water quality (e.g., trace element measures) and fish communities, which involves the archiving of fish age structures (i.e., otoliths). Fish otoliths are metabolically-inert, calcified structures used to determine trace element exposure history through the characterization of element concentrations in the annuli. The CAMP program allowed us to test several hypotheses, specifically that the detection and correlation of water and otolith trace elements is related to geology underlying waterbody, waterbody impoundment, and species. In this study, CAMP water quality data from 2008-2014 (for elements Ba, Mn, Sr, Mg, and Na) were compared to otolith chemistry of two commercially relevant fish species, lake whitefish (*Coregonus clupeaformis*) and walleye (*Sander vitreus*) (caught in 2013-2014) from impounded and non-impounded systems (n=4 each). When comparing averaged water and otolith trace element concentrations over the 2008-2014 year range for the CAMP waterbodies it was found that Sr alone showed a positive and significant correlation. Geology was found to play some role in elevated trace element detection in water. Regarding impoundment, it was observed that with greater amounts of impoundment upstream of a waterbody, the greater the detected levels of Sr in the otolith; potentially exposure to more easily weathered rocks containing limestone may be important. Impounded waterbody Ba and Sr levels in otoliths were significantly greater than non-impounded for lake whitefish and Sr alone for walleye. Lastly, walleye were found to take up greater Ba while lake whitefish took up more Na, Mn, Mg and Sr. Our preliminary findings indicate that re-constructing past trace element exposures suggests impoundment may have an effect on Ba and Sr signatures.

Lingering Impacts of Oil and Fuel Spills – Fate and Toxicity of Persistent Hydrocarbons and Polar Metabolites

TP070 A reactive transport model for naturally occurring arsenic at a crude oil spill site

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A 1979 crude oil pipeline rupture spilled 10,700 barrels of petroleum hydrocarbons into an alluvial aquifer near Bemidji, MN. Since the spill, biodegradation of hydrocarbons has been coupled with reductive dissolution of ferric iron (Fe(III)) hydroxides in aquifer sediments. An overlooked consequence of biodegradation and Fe(III) reduction is the mobilization of naturally occurring arsenic (As) sorbed to Fe(III) hydroxides, resulting in elevated As in groundwater. Dissolved As concentrations have been measured as high as 230 µg/L in the hydrocarbon plume. Arsenic exposure above the maximum contaminant level of 10 µg/L for As, has been linked to skin, lung, and bladder cancers. Although aquifer sediments act as the source for As at the Bemidji site, they also act as an As sink via resorption at the leading edge of the plume, where groundwater is suboxic. Even low concentrations of dissolved oxygen can oxidize dissolved ferrous (Fe(II)) in groundwater, creating an "iron curtain" of freshly precipitated Fe(III) hydroxides that sorbs dissolved As. However, sequestration of As in the Fe curtain is not permanent; our data show that as the hydrocarbon plume expanded from 1993-2013, the Fe and As rich curtain migrated ~30 m downgradient. Given the complex biogeochemistry of Fe and As and the considerable mass of hydrocarbons and associated biodegradable organic carbon remaining in groundwater that might spur additional As mobilization, we are developing a reactive transport model to simulate As cycling in the Bemidji aquifer. This model builds upon previous modeling of Fe(III) cycling in the aquifer with addition of a surface complexation modeling component to describe the fate and transport of As at the Bemidji site. New insights from the model are expected to include: 1) identifying specific geochemical mechanisms (i.e., mineral phases) important for As sequestration; 2) quantifying when (decades in the future) all dissolved As will be sequestered by sediments; and 3) quantifying how far downgradient As will be transported over the plume's lifespan. Better knowledge of these three factors will aid in developing effective long-term remediation strategies for organic-contaminated sites. This work highlights that in addition to considering organic contaminants, remediation strategies for petroleum spill sites should consider naturally occurring inorganic contaminants, which can pose additional, and potentially greater, risks to groundwater quality and human health.

TP071 Evolution and Fate of Petroleum-Derived Dissolved Organic Matter at the National Crude Oil Spill Research Site in Bemidji, MN

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Crude oil is a complex mixture predominately comprised of hydrocarbons that are insoluble in water. Once released into the environment, biotic and abiotic degradation processes immediately begin to oxidize these hydrocarbons, transforming them into polar, water-soluble compounds. This petroleum-derived dissolved organic matter (DOM) may then be readily exported from point-sources and transported through aquatic ecosystems. The extent of the environmental impacts on surrounding ecosystems is dependent on the chemical composition, structure and concentration of the DOM. Fortunately, the same biogeochemical processes that transform non-polar hydrocarbons into water-soluble oxidation products also continue to degrade these compounds after they are dissolved in water. Whereas these dissolved organic products may initially be toxic to native flora and fauna, natural attenuation through prolonged biotic and abiotic degradation processes may transform this material to that which is environmentally benign. The National Crude Oil Spill Research Site in Bemidji, MN provides the opportunity to study natural attenuation of petroleum-derived DOM. Here we report the molecular-level transformations associated with biodegradation of the DOM emitting from the north oil body at the Bemidji research site. Advanced analytical characterization techniques reveal that freshly produced petroleum-derived DOM nearest to the source has a unique signature comprised of relatively aliphatic, high H/C, low O/C and blue-shifted fluorescence. However with time, biological processes decrease the H/C and incorporate oxygen heteroatoms into the petroleum-derived DOM, transforming it into that which is compositionally similar to the native DOM in the groundwater. Preliminary toxicity screening measurements indicate that relatively fresh petroleum-derived DOM may be toxic and that the humification process has the potential to completely remediate these compounds. Furthermore, strategies for modeling the chemical transformations of DOM from natural attenuation are discussed. Finally, we show how information extracted from these models may be implemented for in-situ monitoring of natural attenuation and toxicity at sites where gas and oil leaks contaminate surface or groundwater.

TP072 Evidence of potential toxicity of groundwater contaminated by a 1979 crude oil pipeline release

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Published evidence indicates that chemicals found in petroleum may exhibit a variety of biological activities that can lead to adverse health effects. Given the recent intensification of state and federal efforts to consider contaminants of emerging concern (CECs) in drinking and surface waters there is a need to characterize the potential of waters contaminated with petroleum hydrocarbons and their metabolites to interact with biological targets and exert toxicity. We utilized current and historical biogeochemical and chemical data from the well-studied petroleum–impacted groundwaters at the Bemidji Crude Oil Spill Site. Samples of waters from various contamination levels and biodegradation zones within the plume were assessed for: 1) acute toxicity (in situ, raw samples, Microtox®, N=30), and 2) human nuclear receptor (NR) activity (solid phase extracted samples, 48 NRs evaluated using Trans-FACTORIAL™ platform, N=5). Acute toxicity (up to 51%) was observed only in samples with relatively high nonvolatile dissolved organic carbon (NVDOC) concentrations (e.g., 29.7 mg/L C), but not in less contaminated (e.g., 3.3 mg/L C) or background samples (e.g., 1.7 mg/L C). In vitro NR assays indicated upregulation in a few predominant molecular targets including: peroxisome proliferator-activated receptor gamma and alpha (PPAR), estrogen receptor alpha (ER), pregnane X receptor (PXR), and retinoid X receptor beta, (RXR). Increased activation of these pathways may be linked to adverse impacts on development, endocrine and liver function. Higher levels of NR activity were observed in samples taken from methanogenic zones than those from iron-reducing zones within the plume, and no upregulation was observed in background samples. These results expand our understanding of the potential toxicity of petroleum-impacted waters, and have implications for future decisions on the design and implementation of remediation strategies and regulatory action to manage drinking and surface water resources to protect human and ecosystem health.

TP073 A metabolomics evaluation of oxygen containing compounds identified in groundwater from petroleum fuel release sites

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This evaluation focuses on tentatively identified compounds (TICs) of chemical classes known to be in petroleum biodegradation pathways, including alcohols, phenols, ketones, aldehydes, acids, and esters detected in 109 groundwater samples collected at 22 fuel release sites. Well locations were either upgradient from the fuel releases, within the source area, or downgradient but within the metabolite plume. About 60% of the most commonly detected TICs in downgradient wells were also detected in upgradient wells. A majority of these were fatty acids or esters of fatty acids. Of the TICs commonly detected in downgradient but not upgradient wells, over half were alcohols. Cyclic acids were rarely detected. Principal component analysis indicated a continuum of biodegradation between the source area and downgradient samples with the latter becoming more consistent with upgradient samples. TICs were compared to compounds in the food metabolic database FooDB. About 98% of the TIC chemical formulas were in the database. Using the more specific chemical structure, about a 24% of the downgradient TICs and 43% of upgradient TICs were in FooDB. Results were also evaluated relative to published reports on chemical mixtures found as dissolved organic carbon in unimpacted natural waters. Some TICs may not be petroleum degradation intermediates but are compounds that would be synthesized by bacteria growing on any substrate.

TP074 Use of equilibrium passive sampling for measuring bioavailable PAH and polar metabolites in water and sediment

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Passive sampling devices (PSDs) that are allowed to reach equilibrium with water or water-particle slurries have shown promise in estimating the readily desorbable and potentially bioavailable fraction of polycyclic aromatic hydrocarbons (PAHs). To make further progress toward this goal, we measured the equilibrium polymer-water partition coefficients for 85 PAHs and related heterocyclic compounds and for 27 oxygenated- and nitro-PAHs (O/N-PAH) using polyoxymethylene (POM) as a non-depleting sorbent phase. Our measured partition coefficients (K_{POM}) are in good agreement with previously published values for the USEPA "PAH-34" list and our data set extends the values to a much larger and more useful group of PAH and O/N-PAH degradation products. We used both a static-renewal water-only exposure and a sediment-water slurry exposure to measure K_{POM} and found good agreement between the two methods. Our measured log K_{POM} values were linearly correlated to log K_{OW} ($r^2 = 0.96$ for static-renewal experiment and $r^2 = 0.93$ for sediment-water slurry). We then measured dissolved PAH in a sediment-water slurry, using PAH-contaminated sediment from the field, and compared the POM method to solid-phase microextraction (SPME) for measuring freely dissolved PAH. The POM provided data for 67 PAH and O/N-PAH compounds compared to only 23 PAH for the SPME owing to the much lower detection limits of the POM method. Furthermore, to provide toxicological relevance to our chemical measurements, we conducted experiments with creosote contaminated sediment pre- and post-remediation and found, with some exceptions, that the PAH concentrations in sediment-water slurry measured using the POM was well correlated with accumulation in freshwater mussels, providing strong evidence that the POM method measures only the bioavailable fraction of PAH in the sediment. In addition, we found transient production of polar PAH metabolites during bioremediation that was associated with an increase in genotoxicty of the sediment slurry, although the increase in toxicity was low and likely not a cause for concern.

Crumb Rubber in Sports Fields – Advances in Environmental Chemistry and Exposure Characterization

TP075 A Sorptive Extraction System for Measuring Oral Bioaccessibility of Chemicals from Crumb Rubber

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The growing use of synthetic turf fields has given rise to concern over possible human exposure to chemicals contained in the crumb rubber infill used as cushioning in these fields. The complex polymeric structure and chemical composition of recycled tires, used to make crumb rubber infill, makes it difficult to predict the chemicals humans may be exposed to. For this reason, measuring bioaccessibility is an important aspect of evaluating human risk. The design of traditional physiologically based extraction tests limits the measurement of bioaccessibility of a chemical. As a sample is incubated in an artificial biofluid at a physiologically relevant time and temperature, the chemical concentration in the biofluid can become saturated, limiting the rate of mass transfer between the sample and biofluid. In contrast, epithelial cells can actively remove chemicals from biofluids and into the blood stream using the various transport and metabolic functions. This active cellular process decreases the chemical fugacity in the biofluid, removing the potential for solubility limits. We have adapted a solid phase extraction system, called stir bar sorptive extraction (SBSE), to better simulate the natural extraction processes. In SBSE, a stir bar (coated with a sorption phase) is used to continuously mix an artificial biofluid with the sample and to provide a simulated biological sink. As chemicals are released into the biofluid, they are transferred into the solid phase of the stir bar. By continuously removing chemicals from the biofluid, the extraction process remains in a state of dynamic equilibrium allowing more chemicals to be removed from the crumb rubber sample over a physiologically relevant time period. Three exposure pathways will be evaluated using an SBSE protocol specific for each: oral, dermal and inhalation exposure. This report focuses on the oral pathway which was evaluated with artificial saliva, gastric and intestinal biofluids in both fasted-state and fed-state for both the traditional extraction and SBSE methods. Initial results show significant presence of the chemical signature of crumb rubber and as well as volatile organic chemicals, polycyclic aromatic hydrocarbons, and some halogenated chemicals. The bioaccessible concentration of chemicals from crumb rubber will be compared to the total concentration measured using solvent extraction to calculate the bioaccessible fraction of contaminants in crumb rubber.

TP076 Chemical and Physical Analysis Methods for Characterizing Tire Crumb Rubber Used in Synthetic Turf Fields

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Tire crumb rubber from recycled tires is widely used as infill material in synthetic turf fields in the United States. Tire crumb rubber is a complex and potentially variable matrix with many metal, VOC, and SVOC constituents, presenting challenges for characterization and exposure assessment. The material may also weather differently over time on fields, potentially increasing the variability in its chemical and physical properties. As part of U.S. Federal Research Action Plan on Recycled Tire Crumb Used on Playing Fields and Playgrounds, researchers collected tire crumb rubber samples from nine tire recycling plants and 40 synthetic turf fields across the United States. Multiple analytical methods were applied to measure a wide range of chemicals and to assess factors that may affect exposures. Microwave acid digestion of tire crumb rubber with ICP-MS analysis was used to measure 20 elements. Tire crumb rubber was solvent extracted for quantitative analysis of approximately 50 PAHs, phthalates, thiazoles, and other rubber chemicals by GC/MS and LC/TOFMS methods, with suspect screening for many other potential rubber chemicals. Dynamic emission chamber testing was performed using small or micro-chambers at 25° and 60°C. Emissions of approximately 40 VOCs were collected on Carbopack X cartridges with GC/TOFMS analysis. SVOC emissions were collected on polyurethane foam with GC/MS and LC/TOFMS analyses. Non-targeted analyte data analysis approaches were applied to a subset of the VOC and SVOC analyses. To better understand potential bioaccessibility of chemical constituents, extractions for metals and SVOCs were performed using simulated saliva, sweat, and gastric fluids with ICP/MS or GC/MS analyses. Particle sizes were characterized using gravimetric and SEM methods. Moisture content and rubber/sand fractions were measured. Application of this array of analytical methods improves our understanding of the chemical and physical properties of the tire crumb rubber material used on synthetic fields and is an important step in assessing synthetic field user exposures. However, lack of reference materials and differences in methods and performance between laboratories may present some challenges for data comparability and exposure assessment.

TP077 Concern about Crumb Rubber in Dutch Soccer Pitches

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In the Netherlands discussions are ongoing on the exposure of young soccer players to carcinogenic residues from rubber granulates from recycled car tires. Some soccer clubs have already replaced their pitches and other clubs and local communities have forbidden to use the rubber pitches as long as no clear answers can be given with regard to the associated health risks. One artificial-turf soccer pitch requires in total 120 ton of rubber granulate and every year another 300 kg of crumb rubber is added to maintain the field. Recycling of car tires for the purpose of soccer pitches reduces the overall burden of rubber waste on landfills. However, these rubber granulate pitches may cause indirect environmental damage through leaching of contaminants, for example during rainfall. In addition, there is the possible health risk for young players and goal keepers in particular. Some models suggest sufficient safety margins, but many questions are unanswered. We studied toxic effects of 7-days leachates of eight Amsterdam rubber granulate pitches on zebrafish embryos (*Danio rerio*). In addition, we analysed the leachates and the granulate itself for various organic and inorganic contaminants. Also, non-target screening was performed using direct probe coupled to atmospheric pressure chemical ionization-high resolution time-of-flight-mass spectrometry (DP-APCI-HRTOF-MS). Zebrafish were exposed to three different dilutions (100%, 10% and 1%) of the leachate from 4 hours post fertilization (hpf) till 120 hpf. Every day, visual malformations were scored using a bright field microscope. At day 3, 4 and 5, the heartbeat was monitored. At day 5, Cyp1a activity was assessed with fluorescent microscopy following incubation with 7-ethoxyresorufin. At the highest exposure concentration (100% leachate) zebrafish larvae showed a significant reduction in hatching. This caused an increased mortality in fish older than 4 days. Fish exposed to 100 % leachate should a strong Cyp1a induction in the liver. For some samples this was also seen in the 10% leachate. At day 4 of development a significant increased heartbeat was seen for four of the samples. On the following days, no difference to the control were observed. ?17PAHs in the granulate varied from 18 to 40 mg/kg. Pyrene was the most dominated PAH detected with levels up to 18 mg/kg. These levels exceed the EU maximum tolerance limits for PAHs in consumer products. Zn levels in leachates varied from 40 to 80 mg/kg.

TP078 Is crumb rubber a source for POPs in the marine environment?

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In Norwegian coastal communities, rubber microplastic granules (≤ 5 mm in size) derived from discarded vehicle tires are used in large quantities on outdoor synthetic turf sports pitches. Through transport by waste water effluents and terrestrial runoff, these rubber particles are considered a significant source of MPs to the marine ecosystem. In the here presented interdisciplinary project we study the composition, degradation and environmental impacts of these rubber granules from locations in northern Norway and Svalbard. Plastic litter is an important environmental problem, posing a risk for the health of marine ecosystems and human populations relying on marine resources. At present, many tons of rubber particles reach the marine environment via runoff from land to the sea, and may be further transport northwards with ocean currents. Their persistence and residence time in the Arctic marine environment is unknown. These rubber particles pose a potential health risk for arctic wild life through direct ingestion, especially at the base of the marine food chain (Cole et al., 2013; Booth et al. 2016), but may also provide an exposure route for toxic additive chemicals present in tires to marine organisms (Herzke et al., 2015). Furthermore, the rubber particles may act as a vector for other persistence organic and heavy metal pollutants already present in the marine environment. The extent of the problem is currently unknown, especially in Arctic marine environments with cold water temperatures and long periods with limited sunlight. During a 12 months period, rubber crumbs were placed out in the ocean and sampled continuously for the measurement of persistent organic pollutants, metals and additives. First results will be presented under the conference.

TP079 Measurement of emission factors for chemicals released from new and aged synthetic turf

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Laboratory experiments were conducted with synthetic turf panels to (a) identify chemicals present in gas-phase emissions from new and aged material, (b) quantify chemical specific emission factors for turf fields and (c) explore changes in emissions composition and rates with aging of the turf panels and changes in environmental conditions. The test panels used were constructed to represent complete sections of synthetic turf fields including backing material, synthetic turf blades and crumb rubber infill. The panels were assembled in trays with ultra-low sorption coating providing representative infill loading and surface diffusion characteristics for a specific surface area of field. Emission testing was conducted in small chambers with ultra-inert coating under controlled temperature (25 ^{o}C) and relative humidity (50%) with constant air flow (0.86 $m^3/m^2/h$). A long-term aging test (4.5 months) was conducted with four panels constructed from freshly manufactured material with measurement taken at the start, after 2, 4, 6 weeks of testing and again at the end of the test. The emissions profile for fresh panels and artificially aged panels were evaluated and > 40 chemicals were identified. Emissions include solvents, such as methylisobutylketone and trichloroethylene, and rubber-related substances, such as benzothiazole and naphthalenes. Results from the long-term baseline aging profile show that emission rates drop over time for many but not all compounds. The infill material was re-mixed after aging to simulate the impact of activity on fields and the results indicate that mixing of the crumb material did not significantly alter emissions. The tests were repeated with fresh panels using different aging regimes including elevated ozone, temperature (surface and ambient) and light/dark cycles. The accelerated aging did alter the composition and magnitude of emissions for many of the chemicals. Finally, the relationship between emission rates and temperature (ambient and surface) were determined and final results are reported on a field area basis.

TP080 Modeling the environmental fate of organic chemicals released from synthetic turf

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Artificial turf fields have been steadily replacing natural turf fields mainly because of their higher durability and lower maintenance costs. The turf in these fields mainly consists of plastic blades that simulate natural grass and crumb rubber or mixtures of crumb rubber and sand that provides cushion and simulates soil. Crumb rubber is a type of recycled rubber, which is made of car tires after removing the steel and the tire cord. Because of their ubiquitous presence in schools and sports fields, and the nature of their materials, artificial turf fields have raised some concerns among environmental chemists, regulatory agencies, and members of the public about the potential risks they may pose to humans. We constructed a fugacity-based multimedia model that describes the environmental fate of organic chemicals that are relevant to artificial turf in a standard soccer field. Specifically, we evaluated emissions as a function of the chemicals' physicochemical properties and how emissions might change due to increased action on the field (e.g., by players during practice), and due to changes in temperature and water content of the crumb. We compiled a dataset of about 40 chemicals that have been found in emission testing and/or in the air over artificial turf fields (work done by the same group). Among the most predominant chemicals emitted from synthetic turf to the air were methylisobutylketone, benzothiazole, naphthalene and p-xylene. Starting with equal concentrations in crumb rubber, the concentrations of methylisobutylketone and p-xylene in the air reached 0.5 $\mu mol/m^3$, and those of benzothiazole and naphthalene reached 0.02 and 0.1 $\mu mol/m^3$. With an increase in physical activity on the turf, the concentrations of methylisobutylketone and p-xylene in the air increased by 0.34 and 0.37 $\mu mol/m^3$, while those of benzothiazole and naphthalene increased by 0.72 and 0.68 $\mu mol/m^3$. An increase in the turf surface temperature from 5 to 90 ^{o}C resulted in an increase of 0.59 and 0.71 $\mu mol/m^3$ for methylisobutylketone and p-xylene, and of 0.13 and 0.50 $\mu mol/m^3$ for benzothiazole and naphthalene. An increase in the water content of the crumb from 0.1 to 20% v/v resulted in minor changes in the concentrations of the chemicals in the air at 25 ^{o}C. We conducted sensitivity analysis and compared the modeling data with field measurements to evaluate our calculations. The comparison showed a good agreement between the field measurements and the modeling data.

Fate and Effects of Chemicals from Diffuse Sources and Stormwater

TP081 Pharmaceuticals and Other Micro-pollutants in Precipitation and PM10

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Air and precipitation samples were analyzed for 127 pharmaceuticals, personal care products, and other commercial chemicals within the St. Paul/Minneapolis metropolitan area of Minnesota, U.S. A total of 17 chemicals were detected in samples of airborne particulates (PM10), snow, or rain. N,N-diethyl-meta-toluamide (DEET), and cocaine were the most frequently detected chemicals, found in air, rain, and snow samples, while bisphenol A was detected in snow and air. Four antibiotics - ofloxacin, ciprofloxacin, enrofloxacin, and sulfamethoxazole – were detected.

Ciprofloxacin, enrofloxacin, and sulfamethoxazole were found in precipitation, while ofloxacin was the sole antibiotic detected in PM10. The x-ray contrast agent iopamidol and the non-steroidal anti-inflammatory naproxen were detected in snow, while caffeine, nonylphenol and nonylphenol monoethoxylate were detected only in air samples. Benzothiazole, benzotriazole – 4-methylbenzotriazole, 5-methyl benzotriazole, and 5-chlorobenzotriazole were detected in rain and in snow. Concentrations of all chemicals were in the ng/L range for precipitation and in the pg/m^3 range for airborne particulates. The sources of these chemicals to atmosphere are not known. However, fugacity analysis suggests that wastewater may be a source of nonylphenol, nonylphenol monoethoxylate, DEET, and caffeine to atmosphere. The land-spreading of biosolids, known to generate atmospheric PM10, could also account for the presence of these contaminants in air. Micro-pollutant detections in air and precipitation are similar to the profile of contaminants reported in previous studies of regional surface water. This proof of concept study suggests that atmospheric transport of these chemicals may explain, in part, the ubiquity of these contaminants in the aquatic environment.

TP082 Using integrative passive sampling devices to obtain more meaningful and cost effective data on impacts from stormwater runoff

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In many cases stormwater compliance monitoring is labor intensive, expensive, and largely unsuccessful in providing the data needed to support stormwater management goals. In addition, data from manual grab sampling and automated composite sampling are rarely conducted in a manner that provides the information required to identify sources of contamination, evaluate the effectiveness of Best Management Practices (BMPs), and inform effective decision making. Furthermore, monitoring requirements are driven by the need to meet low discharge concentration benchmarks for metals and other constituents in stormwater that do not take into account loading into the receiving waters and results in arbitrary monitoring requirements (monthly or seasonally) that are not tied to the driving forces within the watershed such as hydrology (flow regime), weather (storm events and antecedent dry periods), and upland land use and cover. To help address these issues, passive sampling devices including Diffusive Gradients in Thin films (DGT) for Cu, Zn, Pb, and Ni and Polar Organic Chemical Integrative Samples (POCIS) for a wide range of house hold, personal care, pharmaceutical, and endocrine disrupting compounds are being used to monitor stormwater runoff. In the Puget Sound a network of monitoring stations was established in Sinclair and Dyes Inlets to assess runoff from industrial areas of Naval Base Kitsap as well as commercial, residential, and rural areas within the watershed. At Naval Base San Diego passive samplers were co-located with autosamplers to provide a direct comparison with grab and composite sampling. Preliminary results from multiple DGT deployments showed that time-dependent variability in stormwater impacts on ambient metal concentrations could be detected on small time scales, as well as over multiple days of rainfall. Based on the results from DGTs deployed over different intervals spanning continuous rainfall events of 3-56 days, reproducibility was affected by presence of partially labile complexes, mass loading rate (time to equilibrium) which is proportional to free ion concentration, and variation in resin blank values. Best results were obtained for 3-7 day DGT deployments. The POCIS samplers showed that a wide range of organic compounds could be reliably detected from the surveillance monitoring which should prove very useful for finger printing likely sources of contamination in stormwater runoff in the areas monitored.

TP083 Pollution characterisation of some selected water bodies in Lagos State, Nigeria

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Environmental problems have increased over the last three decades with improper management practices being largely responsible for the gross pollution of the aquatic environment with concomitant increase in water borne diseases and health hazards. Water pollution has been on the increase likewise and this has been found to contribute significantly to environmental problems in many developing countries. A measurable water quality is therefore essential before any meaningful level of pollution can be determined and controlled in such countries. Hence the need for this research which aims at determining the physicochemical charateristics of water samples from selected lagoons in Lagos. This study was carried out using Apapa and Amuwo-Odofin lagoons as case studies. Samples were collected from these lagoons independently.The analysis of the parameters that can be done insitu were done using Lamotte Test Kit (Model AQ-2/AQ-3). The water was fixed using standard reagents for the parameters to be done in the laboratory. The dissolved oxygen, alkalinity, ammonia nitrogen, pH and hardness were someof the parameters evaluated. Data collected were presented as mean and standard deviation and were subjected to one way analysis of variance (ANOVA) to determine the test of significance among treatments. The differences between mean values were evaluated by Duncan's Multiple Range Tests (DMRT). In this study, the average pH was 7.20 for Apapa (T_1) and 6.67 for Amuwo-Odofin (T_2); the salinity recorded varied from 25.22ppt in T_1 to 2.87ppt in T_2; dissolved oxygen 5.33mg/L in T_1 and 3.11mg/L in T_2; alkalinity 84.89mg/L in T_1 and 93.56mg/L in T_2; ammonia 0.01 for T_1 and 0.57 for T_2 and lastly, the nitrite has 0.00 for T_1 and 0.02 for T_2. There were significant differences in all the parameters evaluated for the two sites except for temperature. The pH, temperature, salinity, ammonia and alkalinity of the two sites fell within acceptable limits, the dissolved oxygen for T_1 followed this same trend however this was not so for the T_2 which suggests possible pollution. The water body in Apapa was free of pollution at the time of this study, possibly influenced by rainfall however the Amuwo-Odofin showed signs of pollution. Conclusively, more attention should be given to studies as this for proper information to influence good policies that will improve the health of our water bodies and the planet at large.

The Role of Plasticity in Evaluating the Effects of Environmental Contaminants

TP084 Amphibian response to environmental contaminants: A role for the microbiome?

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Gut microbial communities play critical roles in host metabolism and physiological function and are shaped by host genetics and environmental factors, including the presence of contaminants. Exposure to contaminants in early development is known to alter the gut microbiome and have lasting effects on host phenotype. However, most studies rely on model organisms reared in artificial laboratory conditions that are known to affect microbiomes. In a previous study we bred adult southern toads (*Anaxyrus terrestris*) and reared the larvae through metamorphosis in their natal, trace element contaminated wetland and a wetland with no history of contamination. We examined several life history traits and quantified the genetic variation of and plasticity in these traits. Most traits were heritable, but a substantial proportion of phenotypic variation remained unexplained by parentage or the measured environmental variables. We sought to determine 1) how gut microbial communities differed among toads reared in habitats with and without heavy metal contaminants and 2) whether the composition of these microbial communities was related to variation in life history traits. We examined the gut microbial communities from the digestive tracts that were preserved from the metamorphic toads developing in each environment. We found rearing environment influenced microbial beta diversity but not richness or other alpha-diversity metrics. However, gut microbial richness and diversity were negatively correlated with developmental time. Richness, alpha diversity and beta diversity were all positively correlated with growth and mass at metamorphosis and beta diversity was also positively correlated with developmental time. The relationships between gut community structure and toad developmental rate appear partly due to the relative abundances of several microbial taxa, some of which are known to elicit disease in amphibians and other aquatic vertebrates. Of particular interest is that all relationships between gut microbial communities and host phenotype were dependent on the environmental context in which they occurred. These results suggest the role contaminants play in shaping these communities is complex and that host gut microbial communities could influence plasticity in fitness related traits in a wild population of amphibians. In addition, this relationship further demonstrates why field and lab experiments examining toxicants often yield different results.

TP085 Plasticity in trace and major element uptake provides insight into long-term impacts of a contaminated aquatic environment

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Environmental contaminants are an issue of global concern for wildlife, but it remains challenging to assess the long-term impacts of contaminated environments on wildlife populations. While laboratory studies allow for complete isolation of the contaminants of interest, they can over- or underestimate toxicity due to the artificial conditions. Using individuals from wild, outbred populations and rearing them in the environment of interest can provide a more realistic model to assess the effects of human impacted environments on wildlife. Further, incorporating reciprocal transplant studies can distinguish how populations with different exposure histories respond to environmental pressures. Using this approach, we examined two populations of southern toads: one inhabiting a site impacted by trace element contaminants from coal combustion waste (CCW) for over 60 years and another in a nearby wetland with no history of CCW input. We reared tadpoles from multiple full and half sib families from both populations to metamorphosis in both habitats. Our results indicate that these populations have diverged phenotypically in a pattern consistent with local adaptation to CCWs. To link this phenotypic change directly to contaminant tolerance, rather than other aspects of the aquatic environment, we analyzed the total body burdens of trace and major elements in the metamorphic toads. We found these populations differed in trace and major element homeostasis when reared in the CCW-impacted environment. Individuals from both populations accumulated greater levels of trace elements in the contaminated environment. However, examining plasticity in accumulation between populations indicates the populations differ in their physiological response to contaminant exposure. Acute toxicity to certain metals in aquatic organisms is primarily due to a net loss of sodium across the gills that results in a physiological cascade ending in death. Whole body sodium levels were significantly reduced in the naïve population reared in the CCW-environment relative to the CCW-tolerant population and those developing in the absence of CCWs, suggesting phenotypic divergence between populations may be due to evolved physiological differences in metal tolerance. By incorporating phenotypic plasticity we were able to provide further evidence of adaptation to a habitat impacted by multiple contaminants, as well as a potential mechanism by which that adaptation may have occurred.

One Health – Opportunities for SETAC Leadership in Integrating Environmental, Human and Animal Health

TP086 Representing Causal Knowledge for Integrated Management with Qualitative Influence Diagrams

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Designing management interventions for ecological and human health benefits requires causal knowledge of the system that is being intervened upon both before and after intervention implementation. In order to appraise and communicate the effectiveness of interventions, a useful conceptual model approach should be flexible enough to accommodate the causal understanding of the intervention impacts on human health and ecological receptors as well as remedial risks; readily adaptable to quantitative and qualitative modeling applications for a variety of disciplines including economic, sociological, and ecological interests; capable of being rapidly developed in a workshop or interview setting for cognitive mapping of key ideas and discussions; helpful in identifying and defining key eco-health system components; able to capture useful details on causal connections between interventions and outcomes; and interpretable by both technical and non-technical audiences A two-phase qualitative influence diagramming approach is discussed that can meet the above requirements and can be used as a focal analytical tool for all phases of the decision analysis process from problem formulation to prediction to adaptive management. Combining logic map applications in the Guide to Community Preventive Services with qualitative Bayesian network applications and environmental frameworks can provide powerful causal representation tools in integrated management. Qualitative influence diagrams can be used to develop decision support tools that examine the evidence base for quantifying risks and benefits in protecting, restoring, and enhancing human health and ecological structure and function.

TP087 Effects of habitat and culture medium on the fatty acid composition of African catfish (Clarias gariepinus)

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Fish is one of the few sources of Poly Unsaturated Fatty Acid, PUFA (especially omega-3) and this makes fish preferable as an animal source protein food. Catfish is the most widely cultured and invariably consumed fish in Nigeria. The fatty acid profile of any food is used as indices of how healthy the food is. There have been so many controversies recently as some scientists claimed catfish has bad fat, working along this area also revealed many variations even among the same specie of fish and many work have been done on the importance of healthy environment but very few on the link with animal and human health. These bought about the quest to find answers to some off these controversies hence the need for this research which aims at assessing the effect of habitat and culture medium on the fatty acid profile of catfish. Fish samples were obtained from two habitats (fresh and brackish water bodies) and from three culture mediums (cage culture, plastic and concrete tanks) from the same location. Lipid in the muscle of the fish was extracted using the Bligh and Dyer method. Extracted lipids were then analysed by Gas Chromatography for the FA composition. Data collected were presented as mean and were subjected to One-Way Analysis of Variance (ANOVA) to determine the differences among treatments. The composition of fatty acid in the samples from the diffrent habitats were significantly different (Saturated Fatty Acid, SFA: 22.86, Mono Unsaturated Fatty Acid, MUFA: 73.62 and PUFA: 3.54; SFA: 33.19, MUFA: 51.47 and PUFA: 15.35 in the fresh and Brackish habitats respectively). The FA profile of plastic tank was significantly different from the other mediums while that of cage culture and concrete tanks were of similar profile (SFA: 25.48, MUFA: 29.89

and PUFA: 44.65; SFA: 32.54, MUFA: 18.92 and PUFA: 49.16 and SFA: 30.03, MUFA: 26.47 and PUFA: 43.50 in the cage culture,plastic and concrete tanks respectively). This suggests that these factors have effects on the FA profile of catfish. From this research it can be inferred that - catfish is an excellent source of PUFA (Omega-3 FA) which is proven to be very healthy and having a controlled and brackish habitat provides a better fatty acid profiled fish. This research also encourage further investigation into the speculative information of the characteristics fatty acid profile in arrays of fishes which will inadvertedly improve empowerment and health of stakeholders and the general populace.

TP088 Increased Schizophrenia-Like Disorders in Carbon Disulfide Exposed Populations Caused by Interference in Norepinephrine Pathway and Production

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Schizophrenia is a serious chronic mental disase affecting more than 2 milling American and over 21 million people worldwide. Due to its capability as a neurotoxicant, exposure to carbon disulfide (CS2) has shown to increase the risk of shcizophrenic-like symptoms (depression, confusion, inability to perform systematic tasks), increased volatility, inability to control anger and increased risky behaviors in individuals with no genetic predisposition. Studies confirmed elevated levels of CS2 in breath samples of Schizophrenic patients. A study by authors found CS2 and 12 other sulfide compounds in elevated concentrations in the atmosphere in residential areas where energy extractio was occurring when compared to national background levels. This study examines an increased risk for reduction of norepinephrine production by alteration of key metabolic pathways by CS2. A literature search was performed using keywords “carbon disulfide” and “schizophrenia/schizophrenic disorders”. Search was narrowed by using specific terms including “Schizophrenia pathogenesis by environmental factors”. In total 233 abstracts were retrieved and topic appropriate articles were retrieved in full. additional papers including laboratory experiments/animal studies, metabolic production of norepinephrine and Schizophrenia were also retrieved. CS2 exposed workers were found to have reduced levels of nitric oxide (NO) synthesis in the brain. CS2 alters production of norepinephrine by causing a deficiency in the rate limiting enzyme dopamine beta hydroxylase. The enzyme deficiency results in abnormal accumulation of dapmine and deficient conversion of dopamine to norepinephrine. Reduced NO levels affects neuroprocesses in the cerebellum, hypothalamus and hippocampus. CS2 is biotransformed into 2-Thiozolidine 4-Carboxylic Acide (TCCA) which is a risk kfactor for schizophrenia by inhibiting antioxidant reactions and phase II biotransformations of lipophilic compounds. An increased potential for schizophrenic-like disorders were found in populations exposed to elevated atmospheric levels of CS2 and sulfide compounds capable of CS2 conversion. Toxicological impact of norepinephrine production increasing the risk for Schizophrenia was shown to occur with exposure to CS2.

Integrated Tools for Improving Environmental Fate and Risk Assessment for Unregulated Contaminants and Their Mixtures

TP089 Characterization of Complex Non-Aqueous Phase Liquids by Nuclear Magnetic Resonance Spectroscopy

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Non-aqueous phase liquids (NAPLs) formed from the pooling of immiscible solvents in the subsurface pose a unique challenge for characterization, risk assessment, and remediation. Until removed or sequestered, the constituents of NAPL will continue to leach into groundwater, posing an ongoing risk to groundwater contamination. At many current and former chemical facilities, the composition of NAPL can be complex, and, for several reasons, is often incompletely characterized. Analysis of NAPL constituents by conventional chromatographic methods is impaired by the high-levels of solvents present, which require dilutions of several orders of magnitude before analysis in order to ensure the safety and integrity of the instrumentation. These dilutions reduce the ability to study the components present in NAPL mixtures at lower concentrations, including pesticides and other hydrophobic compounds that partition into the hydrophobic NAPL substrate. In addition, conventional analytical methods based on the targeted analysis of known or presumed compounds often fail to identify the structures of compounds that are unknown or for which standards are not available, including degradation products. This poster discusses the use of Nuclear Magnetic Resonance (NMR) Spectroscopy as a non-destructive and unbiased analytical tool to assist in the characterization of complex NAPL samples with minimal sample manipulation. NMR has many beneficial attributes that are of use in the characterization of these concentrated, complex mixtures of chemical contaminants, including: the non-selective fingerprinting of all classes of organic compounds present, the ability to quantify constituents without individual standards and calibrations, and the ability to provide structural information on unknown constituents. Advanced multidimensional NMR methods are discussed, including diffusion ordered spectroscopy, that are able to assist in the deconvolution of the overlapping signals in the spectra of complex NAPL mixtures such that the NMR spectra for individual constituents can be isolated.

TP090 Conversion of Estrone to Estradiol in aquatic systems in poultry litter matrix and assessment of changes in its endocrine disrupting potential

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Elevated amounts of estrogens were detected in soil, animal manure and bodies of water at regions near wastewater plants. World’s human population, discharges approximately 30,000 kg/year of natural estrogens (estrone (E1), estradiol (E2) and estriol (E3)) and higher release come from livestock. In the United States and European Union, the annual estrogen discharge by livestock is at 83,000 kg/year, wherein livestock production has been dominated by concentrated animal feeding operations (CAFOs). Poultry farms are of particular concern due to land application of poultry manure as an alternative nutrient source for organic farming. E1 is the primary form of estrogen present in the poultry litter, and is less estrogenic than E2, however endocrine disrupting effects are observed in fish exposed to poultry litter runoff. Previous studies have shown that vitellogenin is detected in male fathead minnows exposed to E1. Our study focuses on the fate of E1 in the poultry litter runoff. A radioactive ^{14}C-labeled E1 was used to elucidate the conversion of E1 to E2 in poultry litter matrix. ^{14}C - E1 was spiked in the matrix of poultry litter and water to mimic runoff from poultry litter applied in farms. A time-course study was conducted by aerating the matrix with humidified air for 30 days. Samples were collected through-out the time course and analyzed for estrogens using radiochromatography. Mass balance study was also conducted by analyzing the CO_2 formed during reaction using liquid scintillation counter. Results show that E1 converts to E2 in the presence of poultry litter, and the estrogenic potential of the aquatic system is also increased. This research is important to fully understand the fate and transport of the most stable form of estrogen, the estrone metabolite, in aquatic systems and in assessing its estrogenic effect on aquatic animals as demonstrated by feminization of male fish.

TP091 Docking based assessment of environmental chemicals and transformation products

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Endocrine disruption by environmental chemicals in water remains to be a real danger given the high sensitivity of the hormone receptors to the smallest concentrations of hormone mimics [1]. Furthermore, it was shown that some water treatments meant to eliminate the chemicals may create even more potent and harmful derivatives [2]. In addition, due to the flexibility of binding sites of targets and similarity of binding sites of different proteins, the environmental chemicals and their transformation derivatives may also bind to several other targets besides the nuclear receptors and metabolic enzymes. Therefore, it is of great significance to extend the evaluation to the derivatives resulting from treatments and transformations, and extend the targets related to endocrine disruption. A docking and structure-based approach in which each activity is represented by multiple structure ensembles of different protein targets was shown to be an economical and feasible way to prioritize chemicals for experimental validation [3-5]. Here we present a systematic workflow of assessment of environmental chemicals based on 3D structure and docking using expanded list of targets and treatment/transformation derivatives. Some examples of successful use of the workflow are shown. We believe that by conducting this workflow of the extended list of chemicals and the extended list of targets, we can have a better assessment of environmental chemicals and have a whole picture of the interaction of environmental chemicals with human and water organisms. References 1. Schug TT, Abagyan R, Blumberg B, et al. Green Chem. 2013 Jan;15(1):181-198. 2. Wammer KH, Anderson KC, Erickson PR, et al. Environ Sci Technol. 2016 Jul 19;50(14):7480-8. 3. Chen YC, Totrov M, Abagyan R. Future Med Chem. 2014;6(16):1741-55. 4. Kufareva I, Ilatovskiy AV, Abagyan R. Nucleic Acids Res. 2012 Jan;40: D535-40. 5. Park SJ, Kufareva I, Abagyan R. J Comput Aided Mol Des. 2010 May;24(5):459-71.

TP092 Integrating chemical and bio-effects monitoring data to prioritize contaminants: Partial least-squares regression and association rule learning

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Chemical monitoring has been widely used to assess exposure to environmental contaminants, but chemistry data alone is often not sufficient for risk assessment as the effects data for many chemicals do not exist. Recent methodological advances in bio-effects screening now allow for concurrent monitoring of a wide array of biological activities. When collected concurrently, chemistry and bio-effects data can enhance chemical prioritization. To prioritize chemicals in 10 randomly selected streams we integrated analytical chemistry data (146 chemicals targeted) and liver transcriptomics data (60K feature microarray) from the adult fathead minnows exposed to the stream waters (48 h, n=7 fish per location). To integrate and analyze chemistry and bio-effects data we used two approaches: 1) partial least-squares (PLS) regression, and 2) association rule learning (AR); AR is a machine learning method that can be used to discover interesting relationships between variables in large datasets. We used AR to identify which pairs of chemicals and genes are associated (interesting rules were identified using the thresholds for support, confidence, and support ratio). The results of PLS indicated that gene expression profiles co-varied with at most seven contaminants including triclosan, metformin and iopamidol. Up to 71% of detected contaminants were not significantly co-varying with changes in gene expression, suggesting they likely were not eliciting measurable effects at these sites. Results of AR identified fifty to hundreds of associations of interest. For example, we discovered that many genes were activated with high confidence and support, when cotinine was present. There were some inconsistencies in the outcomes of the AR and PLS. For example, AR indicated that the rules with iopamidol (the most commonly detected chemical) had relatively high support, but low confidence and lift. The rules with triclosan and metformin had low support, but high confidence and lift. The preliminary analyses indicated that high frequency of non-detects in the chemistry data, wide datasets, and relatively small sample sizes pose main challenges for utility of PLS and AR. We are currently exploring different data censoring approaches to improve non-detects handling, and are working with additional and larger datasets to advance the understanding of the utility of these methodologies for the chemical prioritization in complex mixture scenarios.

Whole Effluent Toxicity Testing – A Science Evolving – Perspectives, Alternatives and Regulatory Limitations

TP093 Ceriodaphnia dubia Chronic Toxicity Test Variability: Evaluation of Food as a Source of Test Variability

B.C. Jorgenson, S.L. Clark, A.M. Briden, R. Ogle, J.S. Cotsifas, Pacific EcoRisk

Multiple inter-laboratory variability studies have identified significantly different outcomes for split samples tested using the chronic *Ceriodaphnia dubia* reproduction and survival test (EPA 1002.0). The lack of predictable outcomes with spiked samples that should be toxic and blanks that should not be toxic, as well as inconsistent outcomes for the testing of samples with an unknown level of toxicity, is troubling and results in diminished confidence in the testing protocol by regulated parties. Where the method is required for compliance determination, such variability results in ambiguity for regulatory staff. The specific driver(s) for such variability remain to be fully understood, but there is considerable flexibility in the method for laboratories to perform the test in different ways, including the selection of food for culturing and testing. The method recommends that the use of a combination of yeast, Cerophyll$^{\text{Ó}}$, and trout chow (YCT), along with the unicellular alga, *Selenastrum capricornutum* (now *Raphidocelis subcapitata*). Laboratories may prepare these foods 'in-house' or use commercially-prepared products provided by an external vendor. Laboratories may also use other algal species, other substitute food combinations (such as fish flake food), or different feeding rates as long as test performance criteria are met and side-by-side comparison tests confirm acceptable quality. For each batch of new food received by or prepared by our laboratory, a side-by-side test with a batch of food that has previously passed our quality control criteria is performed for 14 days (i.e., the window of time organisms are used in cultures). In this poster, we present the outcome of comparative testing of multiple food sources, as well as the effects of food on culture performance, and link this data back as possible sources of variability that have been observed in inter-laboratory comparison studies.

TP094 Ceriodaphnia dubia Chronic Toxicity Test Variability: Evaluation of Water as a Source of Test Variability

A.M. Briden, S.L. Clark, B.C. Jorgenson, R. Ogle, J.S. Cotsifas, Pacific EcoRisk

Multiple inter-laboratory variability studies have identified significantly different outcomes for split samples tested using the chronic *Ceriodaphnia dubia* survival and reproduction test (EPA 1002.0). The lack of reliable outcomes with spiked samples that should be toxic and blanks that should not be toxic, as well as that inconsistent outcomes for the testing of samples with an unknown level of toxicity, is troubling and results in diminished confidence in the testing protocol by regulated parties. Where the method is required for compliance determination, such variability results in ambiguity for regulatory staff. The specific driver(s) for such variability remain to be fully understood, but there is considerable flexibility in the method for laboratories to perform the test in different ways, including the selection of laboratory control water for culturing and testing. Per method guidance, an acceptable dilution/control water is one which is appropriate for the objectives of the test; supports adequate performance of the test organisms with respect to survival and reproduction; consistently meets test acceptability criteria; is of consistent quality and does not contain contaminants that produce toxicity. For tests performed to meet NPDES objectives, the test method recommends that synthetic moderately hard water should be used. In the nearly 25 years that our company has performed this method, we have performed extensive evaluations of various types of water to determine the role of control and culture water in test variability and culture health. In this poster, we present the outcome of comparative testing of multiple water types as well as the effects of different waters on long-term culture performance, and link this data back as possible sources of variability that have been observed in inter-laboratory comparison studies.

TP095 Culturing and Feeding Evaluation of the Daphnia magna Four-Day Survival and Growth Test Method

D. McCausland, GEI Consultants, Inc. / Ecology; N. Love, S. Skigen-Caird, GEI Consultants, Inc.

In 2009 James Lazorchak, Mark Smith, and Herman Haring presented a new test method based on the growth endpoint for the *Daphnia magna* species. The *Daphnia magna* four-day survival and growth test method was developed to provide an additional option to evaluate the potential for sublethal toxicity in the aquatic environment (Lazorchak, 2009). Current methods for the species involve an acute exposure of only 24 to 96 hours, or long-term chronic exposures of 21 to 28 days. The proposed method would allow a shorter duration test while still incorporating the sublethal endpoint. Recently, many dischargers in the Western United States have been struggling to meet whole effluent toxicity (WET) sublethal limits for *C. dubia* due exclusively to interference from the total dissolved solids (TDS) components in their effluent. While the four-day *D. magna* method has not been approved by the United States Environmental Protection Agency, we have begun to evaluate concurrent chronic *C. dubia* and four-day *D. magna* tests in various waters in Colorado to determine whether the method could potentially isolate the TDS component while still providing an estimation of true toxicity. At the 2016 SETAC conference in Orlando, we presented several key observations and suggested modifications to the method focusing on control performance criteria requirements. This current study focuses on nutrition and culture practices to eliminate factors that may be affecting the reproducibility and test performance. Recommendations will be made to streamline *D. magna* culturing practices and components of the test method to reduce variability.

TP096 Daphnia heart beat as a fast toxicity screening measure for industrial wastewaters

S. Park, J. Park, Seoul National University Graduate School of Public Health; K. Choi, Seoul National University / Environmental Health Sciences

Industrial wastewater contains numerous chemicals that can affect integrity and sustainability of aquatic ecosystem. Several countries have developed effluent discharge control measured based on ecotoxicity. This measure includes acute and chronic toxicity tests employing indicator species representing each trophic levels. However, these tests often take long time and may not be appropriate as fast screening tools. In the present study, we adopted heart beat of *Daphnia magna* and investigated its utility as a fast toxicity screening tool for major industrial wastewater effluents. First, we chose two major industries of which toxicant discharges are of concern in Korea, i.e., metal plating, and semiconductor industry. Based on literature search, we identified several typical contaminants of the study industrial wastewater, which include Cu, Cd, and Cr. Following exposure to the target metals, Daphnia heart beat rates decreased in concentration dependent way. Effective concentrations based on heart beat rates were comparable to those derived from D. magna 48 hr immobilization test (OECD TG 202) on copper sulfate ($CuSO_4$) and potassium dichromate ($K_2Cr_2O_7$). Cu showed more severe effects on heart beat rates compared to Cr. Second, we selected cosmetic industry in consideration of organic chemical toxicity impact too. The target organic chemicals are some phenolic compounds, i.e., di-ethyl hexyl phthalate, nonylphenol and octylphenol. We conducted both toxicity tests of target organic chemicals for larger application of fast toxicity screeing tool. In conclusion, not only the experiment rapidity but also sensitivity of test results can be are provided. Thus, reliability of fast screening method is proved and this can be applicable to the rapid effluent toxicity assessment.

TP097 Evaluation of the toxicity of an effluent entering the Deep Fork River, OK

J.K. Kunz, N. Wang, J.A. Steevens, USGS / Columbia Environmental Research Center; S. Dunn, D. Martinez, US Fish Wildlife Service

Effluents from a local manufacturing company were suspected as the cause of two mussel kills in 2005 and in 2011 in the Deep Fork River (DFR), OK. Previous studies reported elevated potassium in water contaminated by the effluent. The objectives of this study were to (1) assess the potential toxicity of the effluent to a unionid mussel (*Lampsilis siliquoidea*) and two commonly tested species (cladoceran *Ceriodaphnia dubia*; fathead minnow *Pimephales promelas*) in 7-day effluent bioassays; (2) evaluate the relative sensitivities of the three species to potassium in 7-day toxicity bioassays; and (3) determine the influence of water quality characteristics on the acute toxicity of potassium. Results of the toxicity studies indicated that the effluent-contaminated water contained 50 times more potassium than upstream DFR water and was highly toxic to mussels and cladocerans. The mussel was more sensitive than the cladoceran and fathead minnow in all tests. The acute toxicity of potassium to the mussel and cladoceran did not substantially change in four reconstituted waters mimicking water quality characteristics in the DFR with a broad hardness range of 35 to 300 mg/L as $CaCO_3$, and importantly, the 50% effect concentrations for mussels were below the potassium concentrations measured in the DFR.

TP098 Ceriodaphnia dubia Chronic Toxicity Test Variability: Is There a Need for Method Improvement?

S.L. Clark, B.C. Jorgenson, A.M. Briden, R. Ogle, J.S. Cotsifas, Pacific EcoRisk

Since 1985, USEPA has released four editions of the testing manual for estimating thechronic toxicity of effluents and receiving waters to freshwater organisms, with the last version of the manualbeing released in 2002. Each revision to the manual has included improvements to the methods, includingculturing, test conditions, and quality control, based on increased experience of laboratories performing thetesting, while still providing laboratories with flexibility for a variety of test method elements. During the1980s and 1990s when the methods were undergoing revisions, there was a collaborative atmosphere amonglaboratories that were performing the methods, including the formation of the Northern California and SouthernCalifornia Toxicity Assessment Groups (NCTAG and SCTAG) where laboratories shared their experienceswith the methods. However, there has been little collaboration among laboratories working on these methodssince then, even though there is a growing body of data that suggests that there is further need for methodimprovements. For example, several recent publications have indicated that the results of tests using thechronic Ceriodaphnia dubia survival and reproduction test can vary significantly among laboratories. Themagnitude of this issue was further demonstrated in an inter-laboratory comparison study coordinated by theSouthern California Coastal Water Research Program in 2015/16, in which the chronic Ceriodaphnia dubia testwas identified as the most variable method in their study. The result of such variable test results is diminishedconfidence of the regulated community that is required to use this method for their compliance determinations.Where the method is required for compliance determination, such variability results in ambiguity for regulatorystaff. This has been further exacerbated by parties that compare results from two different laboratories withoutthe use of referee laboratories to resolve how to address results should they differ between labs. In this poster,we provide chronic Ceriodaphnia dubia test data from multiple inter-laboratory studies, propose potentialdrivers for variability in the outcomes of such studies, and encourage greater dialogue and studies amongexperts in the field so as to increase the confidence of both the regulated community and regulatory agenciesthat require the method for compliance determinations.

Fate and Effects of Metals – Mechanisms of Toxicity

TP099 Amphipods (Hyalella azteca) from populations that are chronically exposed to polymetal mixture exhibit seasonally-dependent tolerance to aqueous Zn

J. Joyner-Matos, C. Higbee, J. Clinkenbeard, C. Davies, G. Davies, B. Goodson, L. Johnston, J. Kenney, E. Knudson, A. Shultz, S. Strange, B. Wolkenhauer, Eastern Washington University / Biology

A century of mining activity in the Coeur d'Alene, ID (CDA) region discharged an estimated 56 million metric tons of tailings into the CDA drainage, including the CDA River and the "chain" lakes in its floodplain. We are exploring whether the amphipod *Hyalella azteca* can serve as a sentinel for the mining-impacted chain lakes. We compared the Zn tolerances of amphipods from four chain lakes that contain metal-rich sediment to those of amphipods from an unimpacted lake and from a commercial source. Zn doses used in the water-only, 96 h toxicity tests ranged from 0 mg/L to 58 mg/L (hardness, 105-110 mg/L as $CaCO_3$). We assessed amphipod survival daily and measured the swimming activity of amphipods at the end of the toxicity test. Amphipod survival in the control was at least 90% in all tests. As the swimming activity of amphipods from all populations was severely impaired by all Zn doses, we could not use this sublethal metric to test hypotheses about metal tolerance. The ability of the field-collected amphipods to survive the Zn exposure varied significantly across season (June, August, and October). Commercially-obtained amphipods, tested only in the spring, had lower survival at all Zn doses than did the amphipods collected from the metal-impacted lakes. The amphipods from the reference (unimpacted) lake had significantly higher survival than did the commercially-obtained amphipods, but only at the lower Zn doses. Although the survival of amphipods from each of the chain lakes varied significantly with Zn dose, at least some amphipods survived the full 96 h at the highest Zn dose, which confirms the elevated Zn tolerance of these chronically-exposed amphipod populations. Differences in survival between amphipod populations from the chain lakes and those from the reference lake were not consistent across season, highlighting the importance of including field-collected reference populations and of conducting tests in multiple seasons when characterizing the metal tolerances of wild populations.

TP100 Contribution of dissolved organic matter composition to the dissolved gaseous mercury production in lake waters

J. Yang, S. Han, Gwangju Institute of Science and Technology / Environmental Science and Engineering

The formation of dissolved gaseous mercury (DGM) in surface water is attributable to the photochemical process mediated by dissolved organic matter (DOM). The aims of this study are: 1) to measure reduction (k_{re}) and oxidation (k_{ox}) rate constants and 2) to correlate redox rate constants with DOM compositions of surface waters to understand the mechanisms of DGM production. Three target monitoring sites were chosen based on the result of the self-organizing map constructed with lake water quality parameters: Jangsung Lake is an oligotrophic lake surrounded by forest (85% of catchment area), Kumho Lake is a mesotrophic lake located by the coastal sea, and Yeongsan Lake is an eutrophic lake located downstream of Gwangju city. The composition of fluorescent DOM in surface water was analyzed using the excitation-emission matrix (EEM) fluorescence combined with a parallel factor (PARAFAC) model. The EEM-PARAFAC identified three components of DOM in surface lake waters: humic-like (C1), tryptophan-like (C2), and reduced quinone like (C3). Seasonal monitoring results showed that surface water DGM was highest in the oligotrophic Jangsung and lowest in the eutrophic Yeongsan Lake. The site trend of DGM agreed well with that of the net reduction rate: k_{re}/k_{ox} was highest in the JSL and lowest in the YSL. The linear regression analysis demonstrated a significantly positive correlation between k_{re} and %C3 (r^2=0.92, p=0.01) and a significantly negative correlation between k_{re} and %C1 (r^2=0.93, p=0.008), while the correlation was not clear between k_{ox} and %C3 (r^2=0.59, p=0.22), and between k_{ox} and %C1 (r^2=0.71, p=0.11). The production of DGM might be promoted by reduced quinone-like DOM in oxic lake waters. Currently, we are carrying out laboratory experiments to verify this hypothesis.

TP101 Effect of inorganic chemicals to Daphnia correspond to mineral concentrations of culture medium

T. Abe, National Institute of Technology, Tsuruoka College / Creative Engineering

Various inorganic chemicals exist in environment, their come from not only natural source but also artificial source. Inorganic chemicals include metal, metal ion, metal salts, and nano materials. We expect that the metal salts also affect organisms. The following studies mainly use metal salts as inorganic chemicals. Median effective concentrations (EC50s) were used from previous studies and several literatures. Daphnia magna immobilization test is listed in OECD Guidelines for the Testing of Chemicals (TG202). Acute EC50s of alkali metal salts employed values of metal chloride, metal nitrate and metal sulfate. Following listed compounds. Lithium chloride (LiCl), sodium chloride (NaCl), potassium chloride (KCl) rubidium chloride (RbCl) and Cesium chloride (CsCl) were used for metal chloride. Lithium nitrate (LiNO3), sodium nitrate (NaNO3), potassium nitrate (KNO3), rubidium nitrate (RbNO3) and Cesium nitrate (CsNO3) were used for metal nitrate. Lithium sulfate (Li2SO4), sodium sulfate (Na2SO4), potassium sulfate (K2SO4), rubidium sulfate (Rb2SO4) and Cesium sulfate (Cs2SO4) were used for metal sulfate. M4 medium include following listed compounds; lithium chloride (LiCl), sodium hydrogen carbonate (NaHCO3), sodium metasilicate nonahydrate (Na2SiO3 9H2O) sodium nitrate (NaNO3), sodium bromide (NaBr), disodium molybdate dihydrate (Na2MoO4 2H2O), EDTA disodium salt dihydrate (C10H14N2Na2O8 2H2O), potassium chloride (KCl), potassium dihydrogenphosphate (KH2PO4), dipotassium hydrogenphosphate (K2HPO4), potassium iodide (KI) and rubidium chloride (RbCl). In acute EC50s (Li-, Na-, K-, Rb-, Cs-), M4 concentration (mg / L and mmol / L) have good correlation to -Cl, -NO3, and -SO4 (r = 0.994 - 0.99997). These results indicate that Daphnia acquired tolerance to minerals.

TP102 Selenomethionine increases arsenic accumulation and toxicity of arsenic in rainbow trout (Oncorhynchus mykiss) during chronic dietary exposure

A. Jamwal, University of Saskatchewan / Biology; T. McDonald, University of Saskatchewan / Geology; Y. Saibu, University of Saskatchewan / Biology; G. George, I. Pickering, University of Saskatchewan / Geology; S. Niyogi, University of Saskatchewan / Biology

Selenium is known to influence the toxicity of arsenic (As) in animals, although the precise mechanistic underpinnings of this interaction are poorly understood. We investigated the effects of different dietary selenium levels [as selenomethionine; 1.8µg/g (control), 10µg/g and 40µg/g diet] on the toxicity of dietary As (as arsenite; 80µg/g diet) in rainbow trout over an exposure period of 28 days. Fish treated with As alone showed an increased hepatic lipid peroxidation and a concomitant decline in thiol ratio (GSH:GSSG) relative to the control fish. Interestingly, fish fed with low (10µg/g) or high (40µg/g) concentration of dietary Se in combination with As showed an even higher degree of hepatic lipid peroxidation and a further decrease in thiol ratio relative to the fish treated with As alone Furthermore, our study also revealed that fish co-treated with As and Se (both at low and high levels) accumulated significantly higher levels of As in target tissues (liver, kidney and muscle) relative to fish treated with As alone. Similarly, the synchrotron-based X-ray fluorescence imaging analysis suggested a dose-dependent increase in the co-localization of As and Se in fish brain in fish co-treated with dietary As and Se, whereas no As deposition in the brain was recorded in fish treated with dietary As alone. These observations suggested that Se facilitates As deposition in the brain and likely in other tissues, possibly via bio-complexation. Overall, our findings indicated that elevated dietary Se can incraese tissue-specific accumulation as well as the toxicity of As in fish.

TP103 Speciation of copper, silver, cadmium, and zinc in exposure medium and their toxicity and bioavailability in rainbow trout gut cell line (RTgutGC)

M. Ibrahim, D. Oldham, M. Minghetti, Oklahoma State University / Integrative Biology

To understand the toxicity of metals in the aqueous environment, we need to consider the interactions occurring in the exposure medium as well as the interaction occurring in the cell. Different metals exert different toxicity to organisms. These differences could be related to the metal species forming in the aqueous environment that alters metal bioavailability. This concept is at the base of the Biotic Ligand Model (BLM) used by the EPA to predict metal toxicity in the aquatic environment. The BLM prediction of toxicity is based solely on ionic metal species. However, this model fails to explain the toxicity of neutral and charged metal complexes. Indeed, it has been shown that negatively charged silver species could also be bioavailable and toxic. To investigate the interaction between different metal species and the cell, we have used an in vitro model of the intestinal epithelium of rainbow trout (*Oncorhynchus mykiss)*,the RTgutGC cell line. Visual MINTEQ, a chemical equilibrium software, was used to determine the chemical speciation of silver (Ag), copper (Cu), zinc (Zn), and cadmium (Cd) with components of exposure medium. The exposure medium (L-15/ex) contains the same salts, galactose, and pyruvate as Leibovitz's L-15 medium but no amino acids or vitamins. Effective concentrations causing 50% effects (EC50) were measured using a viability assay based on two endpoints: metabolic activity and cell membrane integrity. Metal uptake and bioavailability will be determined by ICP-MS and measurement of Metallothionein mRNA levels, respectively. Chemical speciation data showed that dominant species in L-15/ex were Zn^{2+}, $CuHPO_4$, $CdCl^+$, and $AgCl_2^-$. According to the calculated EC50s, we have seen a hierarchy of metal toxicity: Ag > Cu > Cd> Zn. Our data suggests that it is not only the ionic form that is toxic to the cell, on the contrary, neutral and charged species can be toxic too. Therefore, further studies are required to find the specific bioavailability and toxicity of different metal complexes.

TP104 Transcriptomic responses of in situ exposure Clam and their associations with metal subcellular partition: Implications for mechanisms of toxicity

Z. Wang, Key Laboratory of Urban Environment and Health

In this presentation, relevant scientific issues regarding transcriptomic responses and subcellular distribution with special emphasis on their associations were investigated through powerful experimental designs and field-based manipulations of in situ exposure. The Clam *Ruditapes philippinarum* is a good biomonitor/bioindicator to marine metal pollution tolerating manifold anthropogenic stressors and is frequently used in aquatic toxicology. However, the molecular mechanisms in response to field metal stress remains largely unclear. A systematic study of transcriptome may provide insights into the mechanisms of acquired pollution tolerance, where high-throughput sequencing is a helpful tool to analyze the molecular response of toxicological effects to contamination challenge. In this study, we investigated the transcriptomic profile of *R. philippinarum* via digital gene expression analysis. Our results revealed a toxicant-dependent pattern of global transcriptional responses, where a total of 92,236,247 reads were produced from two groups (control and in situ exposure) by whole transcriptome sequencing (RNA-Seq). Gene ontology (GO) and kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway analysis were then used on all genes to determine the biological functions and processes. Genes involved in various molecular pathways of toxicological effects were enriched. The differential expression genes (DEGs) were related to stress response and innate immunity. Quantitative real-time PCR (qRT-PCR) measured expression levels of different genes confirmed through the DGE analysis. This study indicates that RNA-Seq for transcriptome profiling can better understand the toxic effects. Furthermore, it also suggest that RNA-Seq is a superior tool for generating novel and valuable information for revealing the toxic effects caused by field exposure at transcriptional level. Overall, the associations not only revealed the fates of accumulated metals, but scientifically favored an improved understanding of toxic effects in response to subcellular level, supporting the focus of metabolic availability on the intracellular processes or events occurring within organism and revealing a cascade of gene expression pattern in the response of *R. philippinarum* to field metal stress and may be beneficial for further analysis of novel environmental metrics. (The author acknowledge financial support by Grant No. 21377125/B070403 from National Nature Science Foundation of China)

SETAC North America Focused Topic Meeting – Risk Assessment of Chemical Mixtures

TP105 Outcomes from the SETAC Focus Topic Meeting on Risk Assessment of Chemical Mixtures

E.J. Van Genderen, International Zinc Association / Environment & Sustainability; M. Ottinger, University of Houston / Animal and Avian Sciences; T. Backhaus, University of Gothenburg / Biological and Environmental Sciences

SETAC has held several events (e.g. SETAC Pellston Workshop®, SETAC Special Science Symposium, EFSA Colloquium) with the aim of reviewing the current state of the science and approaches for establishing mixture risk assessment and chemical management. Following from these foundational steps, a recent SETAC Focused Topic Meeting (FTM) was held on Risk Assessment of Chemical Mixtures (September 2017) to evaluate the interplay between scientific knowledge on the environmental hazard, exposure and risk of chemical mixtures and its consideration in chemical regulation and management. The meeting provided perspectives across chemical class, biological levels of organization, and facets of the risk assessment paradigm. Highlights from the meeting, including a regulatory world tour, novel tools for assessment and interpretation of risk for chemical mixtures, and applications in field settings will be discussed.

Assessing the Role of Contaminants in the Decline of Prairie Complex Pollinators

TP106 Assessing risks of insecticides used in corn and soybean production on monarch butterfly (Danaus plexippus) larvae

N. Krishnan, Iowa State University / Entomology; K. Bidne, R.L. Hellmich, US Department of Agriculture; J.R. Coats, Iowa State University / Entomology; S.P. Bradbury, Iowa State University / Natural Resource Ecology and Management

Over the last two decades populations of monarch butterflies in North America have declined significantly. Conservation efforts in the U.S. Midwest are focused on restoring milkweed (*Asclepias* species), the sole food source of monarch larvae. Restored milkweed habitat could be placed in close proximity of corn and soybean fields, where insecticides are often used for pest management. Risks of monarch larvae exposure to these insecticides at the individual habitat patch and landscape scales are unknown. Larvae could be exposed through the cuticle by spray drift from foliar applications. Dietary exposure also could occur if monarch larvae ingest milkweed leaves with insecticide residues from spray drift deposition or systemic uptake from treated seeds. Cuticular toxicity studies were undertaken with beta-cyfluthrin, chlorantraniliprole, chlorpyrifos, imidacloprid and thiamethoxam and risk quotients (RQs) were calculated at different distances from treated fields (0, 50, 100 and 125 feet) using AgDRIFT, a spray drift model. For aerial applications, predicted percent mortality for 1st instars ranged from 100% to 44%, 100%

to 7% and 100% to 1% at the edge of field, 50 feet and 100 feet away from treated fields, respectively. For 5th instars, percent mortality ranged from 100% to 0%, 97% to 0% and 82% to 0%. In general, predicted mortality rates were higher for beta-cyfluthrin and chlorantraniliprole and lower for thiamethoxam and imidacloprid. Preliminary acute dietary toxicity results for systemic insecticides are being compared with residue values reported in the literature. Cuticular and dietary neonicotinoid exposures to 5th instars caused them to die at the onset of pupation, approximately 96h after treatment. Prior to pupation larvae behaved normally. This phenomenon occurred less commonly with the other insecticides. The estimated larval survival rates obtained from the deterministic risk assessments are being incorporated into a projection model to predict population responses at the landscape level. These analyses will inform conservation costs and benefits of establishing habitat in areas potentially exposed to insecticides.

TP107 The chronic toxicity of a compound to honey bee larvae can be protective to immature non-Apis bees

D.R. Schmehl, Bayer US / Environmental Safety

Honey bees, as well as many species of wild bees, forage up to several miles away from their home and may be exposed to environmental toxins during foraging. Substantial laboratory data on the honey bee toxicity for both the adult and immature stages of development are required prior to conducting a thorough risk assessment for a pesticide registration. Honey bees are managed globally and are readily available for laboratory testing making them ideal as a surrogate species for use in toxicity tests. These data are considered protective of non-*Apis* bees due to the conservative assumptions integrated into the various test designs. This is especially the case with assessing the chronic toxicity of a compound to a developing honey bee. Individual honey bee larvae are transferred from the hive to a well within a well-plate, and reared to an adult. Each developing larvae is exposed for multiple days to the test compound through both, topical and oral route of exposure. Solubility and chemical fate data ensures that the test compound is bioavaible and stable within the diet throughout the duration of the test. The resulting toxicity test endpoints can be combined with exposure estimates for various species of non-*Apis* bees in order to produce a comprehensive risk assessment for non-*Apis* bees.

TP108 Semi-field Study to Assess Potential Effects of Fungicide Iprodione on Honeybees

B. Sharma, FMC Corporation / Global Regulatory Sciences; C. Berg, Eurofins Agroscience Services, Inc.; M. Hill, C. Bonetti, Eurofins Agroscience Services; G. Mitchell, FMC Agricultural Solutions / Global Regulatory Sciences

An OECD guideline 75 tunnel test was conducted by application of 734.4 g a.i./ha of Rovral® Flo Fungicide (Iprodione) on blooming mustard crop. The major endpoints assessed in the study were honeybee mortality, flight intensity, behaviour of the bees in the crop and around the hive, and condition of the colonies (i.e., colony strength and area of various brood stages and food storage per colony, and assessment date). Additionally, samples of flowers, nectar, and pollen were collected and the level of residues in the bee matrices was determined. Based on residue analysis it was demonstrated that honey bees were exposed to high residues of Iprodione. In the treatment group, the residue level in flowers on day one after application was 46080 ppb. Residue levels in pollen samples taken from the pollen traps were at 18533 ppb one day after application. No adverse effects were observed on the overall mortality, flight intensity, behavior, or brood development of honey bees, when compared to control. It is concluded that Iprodione does not adversely affect the health of honey bees when applied in agriculture at commercially relevant rates.

TP109 Effects of Imidacloprid of Honey Bee Gut Microbiota

E. Gain, Middle Tennessee State University / Biology; N.G. Vinas, US Army Corps of Engineers; F.C. Bailey, Middle Tennessee State University / Biology

An emerging area of interest pertaining to colony health is the bacterial community of the honey bee (Apis mellifera) gut. The highly conserved nature of this microbiome suggests it plays a significant role in bee health, possibly conferring nutritional and immunological advantages. Imidacloprid, one of the highest selling insecticides worldwide, is transferred to the nectar and pollen of treated plants and may therefore be ingested by foraging workers. Very little is known about imidacloprid's effects on bee microbiota. The purpose of this in-progress study is to test the hypothesis that exposure to the pesticide imidacloprid alters the community structure of commensal bacteria in the Apis mellifera gut. Live workers are being kept in cages in a controlled lab environment and fed a sucrose solution containing imidacloprid at 5ppb. Following treatment, the relative abundance of gut bacterial taxa in the treatment and control groups will be analyzed using MiSeq 16S metagenomic sequencing and the software Mothur. Additionally, imidacloprid is being assessed for bactericidal activity in vitro on 5 strains of commensal gut bacteria using the broth microdilution method. Results and analyses for both the in vitro and in vivo experiments will be included in the poster presentation.

TP110 Assessing storage patterns and movement of nectar within a honey bee (Apis mellifera) hive

A.J. Krueger, Bayer US / Environmental Safety; A.W. Olmstead, Bayer CropScience / Environmental Toxicology and Risk Assessment

A honey bee colony is a superorganism which stores protein (bee bread) and carbohydrate (nectar/honey) food stuffs within the cells of a hive. Toxicological responses of the colony to dietary contaminants are driven by the distribution of exposures to various honey bee castes and life stages within the colony. Increased understanding of how contaminants partition within the food stores of the hive and the spatial distribution of subsequent residues will improve our evaluation of toxicological responses of honey bee colonies in the field as well as in ecotoxicological studies. To better understand how xenobiotics move in hives following exposure to contaminated nectar, we used food dyes mixed into a supplemented sugar water diet to track movement within hives. Exposure to the dyed sucrose solution matched the 6 week exposure period used in the regulatory colony feeding studies in which treatments are made during a period of nectar dearth in which outside forage is limited. Using UV spectroscopy, we determined the concentration of dye in single uncapped nectar cells from each frame after dividing the frame into eight sections. We found higher concentrations in the super compared to the brood box, suggesting that honey bees treat these food stores differently in proximity to the brood nest. We did not detect a bias either vertically or horizontally across the frames in the honey super. This along with visual observations indicates that uncapped nectar cells are mixed during storage and nectar processing. Results from this work can be used to improve our understanding of honey bee exposure to contaminants within the hive and guide the development of sampling methodologies for hive matrices in regulatory studies.

TP111 Abundance and diversity of non-Apis bees in pumpkin fields with and without an imidacloprid pre-bloom soil application in South Dakota

A.W. Olmstead, Bayer CropScience / Environmental Toxicology and Risk Assessment; K. Huntzinger, Bayer CropScience; J. Louque, Smithers Viscient, LLC / Wildlife

Non-Apis bees provide important pollination services to various crops worldwide in addition to contributing to biodiversity and ecosystem health. A native bee survey was conducted in pumpkin fields as part of a regulatory pollinator field study evaluating potential effects of imidacloprid to bees. Managed honey bee (Apis mellifera) and bumble bee (Bombus impatiens) were placed in study fields. Fields were approximately 40 acres in size each with treatment fields receiving a sub-surface, shanked application of imidacloprid (0.38 lb/A, the maximum labeled soil application rate for cucurbits) at BBCH18 stage. There were five imidacloprid-treated fields and five reference fields investigated. Hand-collected nectar and pollen from pumpkin flowers were analyzed for imidacloprid and the 5-hydroxy and olefin metabolites at three time points during the study. These indicated low levels of total imidacloprid residues with median values in the treated sites ranging from 0.8 to 2.1 ppb for nectar and 3.4 to 7.0 ppb for pollen. Elevated bowl traps at the canopy level of pumpkins were used to survey the bees at the beginning, middle and end of the flowering period in central South Dakota. Nine traps (three blue, three white, three yellow) were placed in four different locations (North, South, East, West) at 25-75 feet from field edges. A total of 5038 non-Apis specimens were collected from reference fields and 6663 non-Apis specimens were collected from treated fields. Specimens were identified to species when possible and a total of 26 genera were represented in the samples. Of those, *Melissodes*, *Agapostemon*, *Lasioglossum* (*Dialictus*), Sphecodes, and Halictus were the most predominant. Trapped bees were most abundant during the beginning and middle of the bloom period, with a large drop off observed at the end of the bloom period most likely due to colder weather and the late season. There were no statistical differences in the total number of trapped bees between untreated and treated fields at any of the sampling time points ($p>0.05$) nor were there differences in Simpson or Shannon diversity indices. Our results indicate that a soil application of imidacloprid did not result any significant impact in the number and diversity of bee visitors to blooming pumpkin fields.

Using Mesocosm and Field Study Results to Set Regulatory Thresholds for Pesticides – Challenges and Opportunities

TP112 Useful lines of evidence for aquatic risk assessment from mesocosm studies with pyrethroids

J.M. Giddings, K. Campasino, Compliance Services International

Over 3 decades, pyrethroid insecticides have been the subject of dozens of mesocosm and microcosm studies, and the reported data continue to provide useful information for aquatic risk assessment. This presentation will review some of the lines of evidence which can inform and support higher-tier risk assessment that can be derived from mesocosm studies using pyrethroids as an example. These include: (1) mesocosm studies consistently demonstrate that pyrethroids are lost from the water column more rapidly than predicted by standard exposure models, largely due to uptake and degradation by aquatic plants and microbial communities, processes not well represented in the standard models; (2) the observed patterns of sensitivity of different aquatic taxa to pyrethroids are quite consistent across mesocosm studies, and also consistent with trends observed in laboratory toxicity studies and quantified by species sensitivity distributions (SSDs); (3) mesocosm studies, unlike nearly all laboratory toxicity studies, allow measurement of effects on whole populations, and also allow characterization of population recovery; (4) since mesocosms provide real-world measures of effect and recovery potential for a given range of loading conditions (total mass of pyrethroid in drift and runoff per hectare of pond surface per year), they provide means of reality-testing whether the risks predicted using standard models for the same range of loadings are realistic or, as is the case for pyrethroids, exceptionally conservative. An approach to weighting mesocosm studies according to objective criteria for relevance and reliability will be applied to the pyrethroid studies.

TP113 Evaluating mesocosm and microcosm studies with atrazine: Interpreting effects and weighting studies for relevance and reliability

J.M. Giddings, Compliance Services International; D. Campana, Compliance Services International / Risk Assessment; S. Nair, Idaho National Laboratory; R. Brain, Syngenta Crop Protection, Inc. / Environmental Risk Characterization

The USEPA has historically utilized different methods to derive an aquatic level of concern (LOC) for atrazine, though all have generally relied on an expanding set of mesocosm and microcosm (“cosm”) studies for calibration. The database of results from ecological effects studies with atrazine in cosms now includes 108 data points and forms the basis for assessing atrazine’s potential to impact aquatic plant communities. Inclusion of the appropriate cosm studies and accurate interpretation of each data point – delineated as binary scores of “effect” (effect score 1) or “no effect” (effect score 0) of a specific atrazine exposure profile on plant communities in a single study – is critical to EPA’s approach to determining the LOC. We reviewed the atrazine cosm studies in detail and carefully interpreted their results in terms of the binary effect scores. The cosm database includes a wide range of experimental systems and study designs, some of which are more relevant to natural plant communities than others. Moreover, the studies vary in the clarity and consistency of their results. We therefore evaluated each study against objective criteria for relevance and reliability to produce a weighting score that can be applied to the effect scores when calculating the LOC. This approach is useful because studies that are more relevant and reliable have greater influence on the LOC than studies with lower weighting scores. When the current iteration of EPA’s LOC approach, referred to as the plant assemblage toxicity index (PATI), was calibrated using the weighted cosm dataset, the result was a 60-d LOC of 20 µg/L.

TP114 Using Higher Tier Data and Models in a Weight-of-Evidence Assessment to Determine a Water Quality Benchmark for Atrazine

D. Moore, Intrinsik, Ltd.; R. Brain, Syngenta Crop Protection, Inc. / Environmental Risk Characterization

Atrazine is a selective triazine herbicide widely used in the United States for control of broadleaf weeds in corn and sorghum. In 2003, the U.S. Environmental Protection Agency (USEPA) concluded that atrazine poses potential risks to sensitive aquatic species, particularly plants. To assess whether measured levels of atrazine could impact aquatic plants in vulnerable watersheds, the Agency needed to determine a level of concern (LOC) below which atrazine would not cause unacceptable adverse effects to aquatic plant communities. Several attempts at developing a community-level LOC have followed from USEPA but none have been formally accepted or endorsed by independent Scientific Advisory Panels. As part of the current registration review process, the USEPA is revisiting development of a community-level LOC for atrazine. This presentation reviews four higher tier approaches that can or have been used for this purpose. Collectively, the methods take advantage of the large number of single species and mesocosm studies that have been conducted for aquatic plants exposed to atrazine. The Plant Assemblage Toxicity Index (PATI) and the Comprehensive Aquatic Systems Model for atrazine (CASMATZ2) incorporate single species toxicity data but are calibrated with micro- and mesocosm study results to estimate community-level LOCs. The Brock et al. (2000) scoring system relies exclusively on mesocosm study results. Lastly, single species toxicity data were used in a modified version of the USEPA’s Water Quality Criteria (WQC) method. The 60-day LOCs calculated using the four methods ranged from 19.6 to 26 µg/L. A weight-of-evidence assessment indicated that the CASMATZ2 method was the most environmentally relevant and statistically reliable method. Using all four methods with weights based on method reliability, the weighted 60-day LOC was 23.6 µg/L.

TP115 Making the most of mesocosms for pesticide regulation: 20 years of lessons learned

M.L. Hanson, University of Manitoba / Environment and Geography

At their core, toxicity tests need to be able to inform ecological risk assessment. This is especially true for cosms. After two decades of working with these systems, a set of three ground rules have emerged to help ensure a successful study that will assist regulators in their decision making process for pesticide thresholds. Simply put, these three commandments are: 1) test plausible hypotheses of effect, 2) ensure robust analytical, and 3) understand the ecology of your experimental systems. Each of these edicts informs the other, meaning the creation of a well-designed cosm study is most definitely a non-linear process. For regulators, the use of cosms should help reduce uncertainty about possible ecological effects, not leave them scratching their heads. Testing pesticide scenarios that are unlikely to happen, not characterizing exposure or chemical fate, and using systems that are unable to support communities that can interact with each other are of little value to risk assessors. This talk will walk you through numerous case studies of where these rules were followed, and where they were not, letting you be the judge as to their value.

TP116 The effects of the herbicide diquat on non-target aquatic biota- a mesocosm study

R.L. Dalton, University of Ottawa / Biology; S. Robinson, Environment and Climate Change Canada / Wildlife and Landscape Science Directorate; V. Sesin, University of Koblenz-Landau / Institute for Environmental Sciences; H. Ben Othman, University of Ottawa / Biology; C. Boutin, Environment and Climate Change Canada / Wildlife and Landscape Science Directorate; A.J. Bartlett, Environment and Climate Change Canada / Aquatic Contaminants Research Division; F.R. Pick, University of Ottawa / Biology

Invasive aquatic plant species are a growing threat to the health of aquatic ecosystems. Consequently, demand for chemical control options is likely to increase as invasive species spread and reach nuisance levels. In Canada, diquat is the only herbicide currently approved for use on submerged aquatic plants and it is also approved for use in some other jurisdictions globally. The objective of this study was to assess the effects of diquat on native and non-native plant communities, algal communities, an amphipod species and an amphibian species using outdoor mesocosms to simulate natural systems. The experimental design included a control and five concentrations of diquat, with five replicates per treatment. Doses ranged from 100% (18.3 L/ha; 1153 µg/L) to 6.4% (1.2 L/ha; 74 µg/L) of the commercial diquat formulation Reward® label rate. Biota were exposed to a single application of diquat for six weeks in 2016. Plants were negatively affected at all concentrations, whereas phytoplankton biomass increased 7 days following application. A clear dose-response was observed for amphipod survival, with a significant decrease at exposure concentrations of 126 µg/L and higher (6-week LC50 = 150 µg/L), whereas diquat had no apparent effect on tadpoles. Subsequent greenhouse experiments indicated that some plant species were affected by diquat at 0.4% of the label rate (4.7 µg/L). A lower label rate, at least in waterbodies with low turbidity, would provide effective control of target species while reducing effects on non-target biota. This higher tier mesocosm approach provided a direct link to current and future regulatory protection goals. Results from this study will inform federal regulators of the potential management options for control of aquatic invasive species, while highlighting some of the challenges and opportunities of using a mesocosm approach to assess the risk that a pesticide poses to aquatic biota.

TP117 Designing an Aquatic Pulse Dose Test with Sensitive Life Stages of Fish

A. Samel, DuPont; M. Teigeler, Fraunhofer Institute for Molecular Biology and Applied Ecology IME / Ecotoxicology; E. Eilebrecht, Fraunhofer IME / Ecotoxicology

Refined exposure tests became part of regulation frameworks e.g. in the EU (EFSA Aquatic Guidance Document 2013). A pulse dose test can be used to address areas of risk that cannot be satisfied with the standard suite of aquatic toxicity tests. The pulse dose considers situations where the expected exposure events in the field are significantly shorter than in the standard laboratory tests. However, the challenge is often to cover exposure profiles from multiple scenarios with one test. Therefore, the maximum exposure (peak) concentration, the number of peaks, the duration of the peaks, and the interval between peaks are considered to simulate a realistic profile covering a large number of scenarios. Each study is very complicated with a unique design, often very expensive, and due to limited experience with a new design there is questionable likelihood of general acceptance by all regulatory agencies involved in the risk assessment (i.e., EU Member States). This makes the study a high risk and high reward test. If it can be shown that there are no impacts on the test fish under the simulated exposure design, an argument can be made for low risk from exposure of the test material to the test organisms. The presentation addresses the many complexities of the study that need to be considered before conducting the test.

TP118 The responses of freshwater organisms to thiamethoxam following acute and chronic exposures under laboratory and mesocosm conditions

L. Baxter, School for Environmental Science; M. Finnegan, Syngenta, Ltd.; D. Pickford, Syngenta / Environmental Safety; J.D. Maul, Syngenta Crop Protection / Environmental Toxicology / The Institute of Environmental and Human Health; S. Emburey, Syngenta, Ltd.; W. Böhmer, Fraunhofer Institute for Molecular Biology and Applied Ecology IME; U. Hommen, Fraunhofer IME; P. Stegger, MESOCOSM GmbH Institut für Gewässerschutz; H. Thompson, Syngenta, Ltd. / Environmental Safety; M. Hamer, Syngenta / Environmental Safety; P.F. Hoekstra, Syngenta Canada / Regulatory and Biological Assessment; M.L. Hanson, University of Manitoba / Environment and Geography

Thiamethoxam is a neonicotinoid insecticide used extensively in agriculture across Canada and around the world. We report the results of extensive laboratory toxicity testing (acute and chronic) conducted under good laboratory practices (GLP) for over 30 freshwater species (insects, molluscs, crustaceans, algae, macrophytes, and fish). Fish and primary producers were insensitive, with acute median effect concentrations (LC50 / EC50) observed to be ≥ 80 mg/L in all cases. Tested molluscs, worms, and rotifers were similarly insensitive (EC50 ≥ 100 mg/L). In general, insects were the most sensitive group, with most acute EC50 values < 1 mg/L. An outdoor mesocosm study was conducted to examine the response of zooplankton, insects, and phytoplankton up to 93 days following a single exposure to thiamethoxam. The No Observed Ecologically Adverse Effect Concentration was 100 µg/L and the $NOEC_{community}$ was 30 µg/L. In addition, a chronic 35-d outdoor mesocosm study was conducted with the mayfly *Cloeon dipterum* using the formulated product. Significant reductions in both larval abundance and adult emergence were observed with a concentration response at 10.0, 3.0 and 1.0 µg/L, with no effects documented at 0.3 and 0.1 µg/L. These findings support a 35-d NOEC of 0.3 µg/L for *Cloeon dipterum* under chronic exposure conditions. Overall, an extensive ecological database exists for use in ecological risk assessments of thiamethoxam.

Risk Communication Toolbox

TP119 Strengths, Issues and Complexities of Risk Assessment and Communication

A. Ethier, ARCADIS

Environmental risk assessment at historically contaminated and/or operational sites is a valuable tool that can be used to evaluate complex and interconnected areas of an ecological system, from source term to receptor. Unfortunately, there are a multitude of discrepancies between environmental monitoring design and risk assessment results, public perception and scientific basis of risk, contaminant screening and risk criteria, among others. The focus of this presentation will be to discuss those discrepancies that have the greatest potential impact on risk perceptions and site management.

Fate of Chlorinated Persistent Organic Contaminants in the Urban Water Cycle

TP120 Evaluation of advanced oxidation process for the elimination of chlorpyrifos in wastewater

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The indiscriminate use of pesticides in the past decades represents serious water pollutants. Chlorpyrifos is a organophosphorus insecticide of broad-spectrum used in agriculture to control pests in rice, cotton and fruit crops. In addition to the management of diseases transmitted by insects that affect livestock breeding, among others. It is also used for the eradication of vectors in the urban areas of the city of Cartagena (Colombia) and its industrial zone (Mamonal). Chlorpyrifos pesticide and its metabolites have been identified in aquatic systems worldwide. It was related to toxicity for aquatic organisms, animals, and humans. Advanced oxidation processes (AOP) are well known for generating highly reactive and non-selective hydroxyl radical species, which are used to degrade most of organics present in water and wastewater. The objective of this study was to evaluate if photo–Fenton (Fe+2/+3/H2O2/UV) process is efficiency in chlorpyrifos removal from aqueous solutions. The catalytic oxidation of chlorpyrifos from synthetic wastewater using Fenton reagent was done at bench-scale in a batch system. The initial and final chlorpyrifos concentration was determined using the gas chromatography technique coupled to electron capture detector and mass spectrometry detectors. Partial results were obtained. The experimental conditions were determined: the dosages of FeSO4 ·7H2O and H2O2, pH the stirring time. The COD values of the synthesized water was 2940 mg/mL and COD removal efficiency of 85.6% with a corresponding effluent COD value of 110 mg/L was achieved under optimal reaction conditions of a pH value of 3.5, T = 25–30 °C, UV lamp=250 nm, a dosage of 2.5 g/L of FeSO4 ·7H2O and 100 mL/L of 30% H2O2 solution, and a stirring time of 6 hours. The Advanced oxidation processes are suitable for the elimination of chlorpyrifos pesticide in contaminated waters.

TP121 PCB cycling in stormwater in an urban high desert: Santa Fe, NM

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Fingerprinting of PCB congener patterns stormwater and surface water from Santa Fe, NM has allowed a rare glimpse of PCB cycling in a desert environment. Santa Fe is classified as a monsoon-influenced dry steppe climate. PCB concentrations in stormwater and baseflow of the Santa Fe River are similar to those observed in other urban areas of the United States, and like these other systems. Lower molecular weight congeners are less abundant in the Santa Fe system compared to cities in temperate climate zones such as New York City, NY; Philadelphia, PA; and Portland, OR. The loss of low molecular weight congeners is not temperature-driven, since the average temperatures are similar in all these cities. Instead, we hypothesize that the low moisture content of both the atmosphere and the soil renders them less able to sorb PCBs. No evidence of microbial dechlorination of PCBs was observed in the storm drains of Santa Fe. The PCB sources in the Santa Fe river basin are dominated by Aroclors, with some non-Aroclor congeners such as PCB 11 present at levels similar to other urban watershed in the United States.

TP122 Whole genome sequencing as a tool to understand reductive dechlorination of tetrachloroethene at an USEPA superfund site

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We have reported on use of whole-genome next-generation sequencing (NGS) as a tool to understand the microbial community response to biostimulation with emulsified vegetable oil (EVO), hydrogen gas and nutrient amendments at the North Railroad Avenue Plume (NRAP) in Espanola New Mexico. This site was designated an USEPA superfund site in 1999 due to contamination with tetrchloroethene (PCE) that threatened this sole-source potable water supply. The published data include samples from 2007, one month prior to and five months after remediation that was sequenced on the Illumina platform and analyzed with Metagenome Rapid Annotation using Subsystem Technology (MG-RAST #mgp 11259). New data includes re-sequencing and analysis of the 2007 sampling events, and extends the findings to samples from 2009 and 2010 for source-area extraction well 3 (SAE3). NGS data for five and 23 months is available for a hot-spot extraction well (HSE6) that was first remediated with whey, then switched to EVO. In addition, 16S ribosomal metagenomes are available for the same time points and for additional wells. As a Joint Genome Institute (JGI) community sequencing project, (CSP 1243), this dataset includes information on viral and CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) sequences. The JGI data confirms and extends the preliminary MG-RAST data; after remediation the community shifts from predominately aerobic to anaerobic species, methanogenic and thermophilic Archaea increase, species capable of dehalogenation increase, and potentially pathogenic bacteria also increase. The whole metagenome data detects 1,440 genera, whereas the 16S metagenome data detects 275 genera. The analysis of genes and functions relevant to the remediation of PCE is underway.

TP123 Cis-dichloroethene degradation in porous media: Biological limitations under different transport conditions

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Bioremediation of chlorinated ethenes in groundwater is an appealing technology that has been applied for over 10 years at contaminated sites. However, under the complexity of transport through porous media, limitations to bioremediation are commonly observed. In this work, we applied a mathematical model to experimental data to understand the biological limitations of reductive dechlorination of cis-dichloroethene in porous media under different flow conditions. The mathematical model accounts for flow and dispersion in the mobile groundwater, mass transfer between the mobile groundwater and a stationary biofilm, diffusion and reaction within the biofilm, and competitive inhibition between cis-dichloroethene and vinyl chloride during reductive dechlorination. Comparing the model predictions to the experimental results permitted the evaluation of three hypothesized biological limitations: insufficient supply of electron donor, decay of dechlorinators' biomass, and reduction in bacterial metabolism rates. Any of these three limitations are able to describe observed experimental data, but insufficient supply of electron donor is the most plausible explanation for failure of dechlorination. Therefore, an important finding of this investigation is that insufficient hydrogen production occurs if the flow through porous media is too slow to provide adequate flux of electron donor. The implication of our findings is that in engineered or natural bioremediation of chloroethene-contaminated groundwater, not only the proper dechlorinating organisms must be present, but also proper groundwater flow conditions must be maintained or else dechlorination may fail.

TP124 Long-term evaluation of source zone bioremediation at chloroethene contaminated sites using passive samplers and historic data

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The objective of this project was to assess the long-term effectiveness of bioremediation in both the source zone and the plume at chloroethene-contaminated sites. Source zone treatment has initially been shown to be an effective approach to decrease contaminant concentration in the plume and decreasing contaminant source DNAPL mass. Although this technology has been widely applied at several sites with initial success, there has never been a thorough assessment of factors differentiating successful and unsuccessful treatment applications, and the economic impact of the treatment technology. Here we evaluate the effectiveness of source zone bioremediation by (1) quantifying how bioremediation treatment strategies have impacted contaminant mass fluxes from the source zone; (2) applied source strength functions (SSFs) to predict source longevity (Power Law Model (PLM) and Equilibrium Streamtube Model(EST)); (3) evaluated treatment effectiveness and monitoring costs at each site. This multi-site study suggests that bioremediation may lead to sustained mass discharge reduction from the source zone and provide a basis for establishing flux-based management criteria for sites treated using bioremediation. SSFs were capable of estimating mass in the source zone and the effect of bioremediation on accelerated removal of contaminant mass compared to if nothing had been done. The cost and performance analysis suggest that treatment may be independent of cost, but bioremediation does have the benefit of sustained removal of contaminants long after active treatment. This meta-study suggests that bioremediation is a successful subsurface bioremediation technology. However, robust post-remedial monitoring plans are needed to properly manage, and intervene when necessary, anaerobic bioremediation efforts.

Development of Biomarkers for Environmental Monitoring, Assessment and Possible Monetary Valuation

TP126 Spatial and Temporal Distribution of PCBs in Soil and Earthworm (Aporrectodea longa) Samples around Electric Power Substations in Benin City, Nigeria

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Polychlorinated biphenyls (PCBs) in top soil and earthworm samples (*Aporrectodea longa*) from electric power substations in Benin City, Edo State, Nigeria, were assessed. Two electric power substations and a control station were chosen to reflect possible hot spots of PCBs pollution. Samples were collected for a period of 6 months (January-June, 2016) and analysis was carried out using Gas Chromatography (GC) equipped with an electron capture detector (ECD). The composition of PCBs homologous in the soil samples was in the sequence: The composition of PCBs homologous in the soil samples was in the sequence: Tetra (48%) > penta (18%) > hepta (13%) > Tri (11%) > Hexa (7%) > Octa (2%) > Di (1%). PCBs congeners profile in soil from the study area was in the order: PCB 60 > PCB 18 > PCB 105 > PCB 52 > PCB 170 > PCB 43 > PCB 180 > PCB 123 > PCB 126 > PCB 128 > PCB 200 > PCB 137 > PCB 185 > PCB 154 > PCB 28 > PCB 77 > PCB 195 > PCB 101 > PCB 7. The mean concentrations of PCBs in soil ranged from 0.001 (PCB 7) to 0.030 (PCB 60), while the mean concentration for the ∑PCBs was 0.087 ± 0.006µg/kg. The mean concentrations of PCBs in earthworm samples ranged from 0 (PCB 185,195,220) to 0.02 (PCB 105). These concentrations were significantly lower than the concentrations in soil (P< 005, F = 15.307). PCB congener profile in earthworm samples was in the order PCB 60 > PCB 105 > PCB 18 > PCB 28 > PCB 123 > PCB 77 > PCB 43 > PCB 52 > PCB 101 > PCB 128 > PCB 7 > PCB 180 > PCB 126 > PCB 195 > PCB 185 > PCB 170 > PCB 137 > PCB 200 > PCB 154, while mean concentration for the ∑PCBs was 0.015 ± 0.003 µg/kg. A significant positive correlation (P< 0.05, r = 0.795) was observed between PCB concentrations in soil and earthworm samples. Spatial variation showed different contamination pattern in the soil and earthworm samples. The highest concentration of PCBs in both soil and earthworm samples were observed in Sapele Road and Ugbowo stations. These areas are characterized by high-to-medium voltage transformer substations implying that that transformer substations could be potential sources for PCB contamination in soil. PCBs levels were also observed to be higher during the dry season months for both soil and earthworm samples. The observed levels in soil samples were generally below the ecological benchmark of 0.3mg/kg, nevertheless, the presence of these toxic compounds in the environment raise concerns because of their persistence and toxic potentials.

TP127 Cytochrome P450 1A expression in Kemp's ridley (Lepidochelys kempii) sea turtle tissues of nesting mothers and hatchlings

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The 3 million barrels of crude oil released into the northern Gulf of Mexico during the 2010 Deepwater Horizon oil spill severely impacted the Gulf's coastal ecology and exposed countless marine organisms, including sea turtles, to oil. The primary habitat of the critically endangered Kemp's ridley sea turtle (*Lepidochelys kempii*) is the Gulf of Mexico, leading to concern for oil impacts on their welfare and conservation. A natural resource damage assessment (NRDA) was performed to evaluate the effects of this spill and appraise future restoration needs. The findings presented here are a part of studies performed for that assessment. Cytochrome P4501A (CYP1A) expression is a known biomarker of exposure to select chemicals, including polycyclic aromatic hydrocarbons present in crude oil. CYP1A protein expression in sea turtle tissue was evaluated via immunohistochemistry. The Kemp's ridley samples included skin biopsies of nesting mothers and paired liver and chorioallantoic membrane (CAM) tissues of dead late stage embryos and hatchlings collected in 2010 and 2011 from Padre Island, TX, USA. All tissues were collected after full emergence of living hatchlings from the nest; within 96 hours of natural death. CYP1A expression was measured following previously published methodology. Tissues were formalin-fixed, embedded in paraffin, and 3-5 µm sections were mounted on microscope slides, prior to staining with a monoclonal mouse antibody to CYP1A. A side study on CAM tissues from Florida loggerhead sea turtle (*Caretta caretta*) hatchlings confirmed the stability of CYP1A protein expression in CAM tissue up to 96h after hatchling emergence. Preliminary analyses of Kemp's ridley samples revealed low CYP1A expression. A moderate positive correlation in CYP1A expression was observed between paired liver and CAM tissues from individual Kemp's ridley embryos, supporting the utility of CAM samples in studies of endangered oviparous species where non-invasive measurement of developmental CYP1A activity is desired.

TP128 Micronucleus cytome assay in the genotoxic and cytotoxic assessment of cadmium and lead in adult Amietophrynus regularis

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In vivo micronucleus cytome (MNcyt) assay is a cytogenetic test for the genotoxic (biomarkers: micronucleus, bud and other nuclear abnormalities) and cytotoxic (biomarkers: binucleated cell, apoptosis and necrosis) monitoring of chemical and physical genotoxins in lower vertebrates. MNcyt was utilized to assess the genotoxicity and cytotoxicity of cadmium (Cd) and lead (Pb) in adult *Amietophrynus regularis*. The 96h acute toxicity of Cd and Pb was determined following *A. regularis* exposure to six concentrations (8 – 512 mg/L) of the metal solutions. Four toads (mean body weight=157±2.16) per group were exposed to five sub-lethal concentrations (5 - 75%) of the 96h LC_{50} of Cd and Pb for 14 days. Similar treatments were given to toads exposed to tap water and Benzene (0.01 mL/L) as negative and positive controls respectively. At post exposure, frequencies of micronucleus (MN) and nuclear abnormalities (NAs) were assessed in bone marrow and peripheral erythrocytes of toads. The tested metals induced concentration and time dependent increase in percentage mortality with 96h LC_{50} for Cd and Pb being 36.36 and 112.06 mg/L respectively. No observable effective concentrations (NOEC); Cd=8.0 and Pb=32 (mg/L) and Lowest observable effective concentrations (LOEC); Cd=16.0 and Pb=64.0 (mg/L) were observed for the metals. Derived toxicity factor (TF) showed that Cd was 3.08 times more toxic to the toads than Pb. The metals induced significant ($p< 0.05$) increase in frequencies of MN and NAs in both bone marrow and peripheral erythrocytes compared to the negative control. Cd elicited 1.42 and 3.26 folds increase in MN and NAs respectively, than Pb. High frequencies of apoptotic and necrotic erythrocytes were observed in Cd treated toads than Pb exposed toads. Increased genetic instability induced by Cd and Pb is associated with genetic related syndromes, neoplasms, reproductive dysfunctions and mortality. This suggests threat to amphibian health and may enhance population decline.

TP129 Biochemical indices and bioaccumulation of heavy metals in snail as biomarkers in risk assessment of exposure to pollution effect of mining activities

C. Ubani, I.N. Onwurah, University of Nigeria / Biochemistry

Artisanal mining is largely a poverty driven activity, and in some parts of Nigeria, it is typically practiced in remote rural areas. Due to the economic recession, the activities of artisanal and small scale miners have substantially increased. The pollution from these mining sites endangers the health of the populace as well as often the surrounding environment. The people living near these mining sites, particularly the miners and their families are exposed to a higher risk. The major pollutants of concern, unlike organic pollutants, are the heavy metals which cannot be degraded. Hence they persist in the environment for years and bioaccumulated in food chain. Consumptions of such contaminated food like snails picked up from these mining-polluted regions elicit reasons for public health concern. Snails are ‘large sinks’ or reservoir of heavy metals accumulated over time and are consumed greatly by majority of the populace. Therefore snails can serve as indicator organisms in an ecosystem such that when they are exposed to environmental pollutants give some biochemical indices that can be meaningfully linked to the ecosystem’s heath. This work quantitatively and qualitatively evaluates the levels of heavy metals in the snail Achatina achatina and the elevation of antioxidant defense system as biochemical biomarkers (enzyme activities) to monitor the impact of the mining activities in selected area. The data generated were modelled to evaluate the health and ecological risk of the exposed population. Human health risk assessment model for estimated daily intake showed a major increase above the Tolerable Daily Intake (TDI) for As, Ni and Pb. Results of Incremental Lifetime Carcinogenic Risk (ILCR) from the human health risk assessment model exceeds the value of the USEPA and were highly correlated to enzyme activities measured in the indicator organism.

The Future is Now – Scientific Strategies for Coping With an Unsteady State

TP130 Challenges to Chemicals Assessment in the Anthropocene

C.A. Ng, University of Pittsburgh / Civil & Environmental Engineering

The concept of a persistent organic pollutant (POP) has been with us for at least three decades, and acknowledgment of the hazard posed by such chemicals prompted the creation and continuing work of the Stockholm Convention. First adopted in 2001, the Convention now has more than 150 signatories and covers 26 chemicals; four more recently underwent review during the POPRC meeting in September 2016. Yet over this same time period, the nature and reach of human-made chemicals has changed substantially. Human influence on the environment has never been greater. As our population and our ability to engineer the world around us grows, humans are now able to effect change on the scale of what used to be called a “natural disaster”—take the recent examples of human-caused earthquakes in Oklahoma as a result of unconventional extraction, or massive coral bleaching events taking place as a result of ongoing climate change. This change in the scale of human impacts on the planet has led to the suggestion that we have entered a new age—the so-called Anthropocene. From the perspective of environmental chemistry, how has entering the Anthropocene changed what it means to be a chemical of concern? Do current formalized risk and hazard assessment procedures and definitions adequately acknowledge the many interacting pressures faced by populations and ecosystems in this strange new world? This talk will focus on three basic components of chemicals hazard assessment—persistence, bioaccumulation potential, and toxicity—as well as the changing landscape of exposure that translates their hazard to risk, within the context of a warmer, more volatile, and increasingly connected world.

TP131 Reducing uncertainty and confronting ignorance about the possible impacts of weathering plastic in the marine environment

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Rockström et al. identified nine planetary boundaries that, if exceeded, could disrupt vital processes that keep Earth in a stable condition. Chemical pollution is one of these nine threats, and has recently been amended to “novel entities” that cover, in addition to chemical pollutants, other substances such as plastic. In previous work, three conditions for a novel entity to pose a planetary boundary threat were identified: (i) the novel entity is causing global exposure that is (ii) poorly reversible and that (iii) affects an Earth system process. Plastic is ubiquitous in the global oceans, and fulfills condition (i) since it is causing global-scale exposure, and condition (ii) since it cannot be readily reversed. Hence, if research discovers that it has a yet unrecognized effect on an Earth system, plastic would represent a planetary boundary threat. This presentation critically reviews unknown effects that might be discovered by studying the sources, transport and weathering processes that affect plastic debris. Weathering of plastic generates microplastic and releases additives, and likely also nanoplastic and chemical fragments cleaved from the polymer backbone. However, plastic weathering in the marine environment is not well understood. Laboratory studies, field monitoring and models of the impact of weathering on plastic debris are needed to reduce uncertainty in hazard and risk assessments. Key research needs are to: (1) improve understanding of the multiple factors influencing the weathering process by characterization of plastic particles and their degradation products over time; (2) characterize how weathering affects the spatial and temporal distribution of microplastic and nanoplastic; (3) identify the adverse effects and mechanisms by which plastic particles and their degradation products affect biological systems; (4) develop and validate standardized test methods suitable for assessing environmental effects of plastic particles and leaching chemicals on model organisms; (5) elucidate the role of biofilms in aggregation, sedimentation, exposure, uptake and effects of plastic particles in marine organisms.

TP132 Taking into account in today's chemicals assessment the legacy of past emissions

M. Scheringer, ETH Zurich / Institute for Chemical and Bioengineering

Production, use and emissions of organic chemicals have increased substantially over the last 50 years. There is a recurring pattern of identification of a contamination problem, phase-out of the chemical(s) causing the problem, introduction of new, but similar chemicals, (exponential) growth of uses and emissions, and again identification of a contamination problem. In particular, this pattern can be observed for several groups of halogenated chemicals, for example for polychlorinated biphenyls (PCBs) and chlorinated paraffins; C8-PFASs (poly- and perfluorinated chemicals) and so-called "alternative PFASs"; and polybrominated diphenyl ethers (PBDEs) and many "emerging" brominated flame retardants. Overall, this has led to substantial emissions of (highly) persistent halogenated chemicals that now form a haze of global contamination. This contamination cannot be ignored in today's chemicals assessment: current emissions of chemicals are not to a pristine environment, but add to a baseline of contamination from (much) earlier emissions. This stock-pollution problem has formed because the persistence of the different types of halogenated chemicals was not appropriately taken into account when these chemicals were admitted to the market. The implication of this situation is that in today's chemicals assessment the weight of the persistence of chemicals in the environment has to be increased. This will make it possible to compensate for the ignored footprint of persistent chemicals from the past. Practically, this could be implemented by reducing the thresholds for classifying a chemical as persistent in assessment schemes for persistent, bioaccumulative and toxic (PBT) chemicals and by introducing a separate category of highly persistent chemicals that are not admitted to the market just because of their high persistence, irrespectively of their toxicity and other properties (obviously, this concept would apply only to low-molecular weight chemicals but not to polymers and inorganic materials).

Environmentally Relevant Behavior Assessment to Support Modeling, AOPs and Improved Risk Decision-Making

TP134 Exploring the effects of temperature and resource limitation on mercury bioaccumulation, growth and energetics, and behavior in Fundulus heteroclitus

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Aquatic ecosystems are affected by changes in both temperature and resource availability. While higher temperatures may result in increased food consumption and increased mercury (Hg) accumulation, they may also increase growth and reduce Hg tissue concentration through somatic dilution. Dynamic energy budget theory provides a broad and generalizable framework based on first principles of energy metabolism that is well suited to understand these interactions, allowing joint acquisition and interpretation of chemical exposure and stressor effect information to be translated into demographic rate changes. In the current study, we conducted growth and bioaccumulation experiments to examine the interaction of temperature and resource availability on Hg accumulation and effects in the estuarine fish *Fundulus heteroclitus* (mummichog). Juvenile mummichog were fed 3.3% or 10% of their dry body weight/day with tuna naturally contaminated with Hg and held at either 15 or 27 °C for 28 days. Growth was low in most treatments, except in fish fed 10% body weight held at 27 °C (40% weight and 12% length increase). Methylmercury (MeHg) accumulation was similar across feeding conditions but increased with temperature (~17-fold increase in MeHg concentration at 27 °C and ~7-fold increase at 15 °C, regardless of feeding rate). In a second experiment, mummichogs from two wild populations with differing native Hg exposures were fed either a high or low MeHg diet. Fish were strip-spawned every two weeks during the feeding period. Adults were sampled for total Hg concentration at the start and end of the experiment, and egg MeHg concentration was measured in unfertilized eggs from each spawning event. Danioscope software was used to assess the heart rate of developing embryos at 10 days post fertilization. Larval behavior was assessed at three and 10 days post hatch using a dark:light movement assay and Ethovision software. Tissue analysis indicated successful maternal transfer of Hg to eggs in the high Hg feed treatment. Heart rate and movement assays indicated potential population level differences in baseline behavior. Overall, this work contributes to the ongoing development of an ecological modeling framework in a fish with an extensive toxicological and genomic background. Ultimately, we are working to connect molecular mechanistic, physiological, reproductive, and behavioral responses to population level fitness.

TP135 Integrative behavioral eco-toxicology (IBET): Bringing together fields to establish new insight to behavioral ecology, toxicology, and conservation

E.K. Peterson, Colorado State University, Pueblo / Communities to Build Active STEM Engagement; C. Ramos, Colorado State University, Pueblo / Biology; J. Swaddle, College of William & Mary / Biology

The fields of behavioral ecology, conservation science, and environmental toxicology individually aim to protect and manage the conservation of wildlife in response to anthropogenic stressors, including widespread anthropogenic pollution. Although great emphasis in the field of toxicology has been placed on understanding how single pollutants affect survival, a comprehensive, interdisciplinary approach that includes behavioral ecology is essential to address how anthropogenic compounds are a risk for the survival of species and populations in an increasingly polluted world. In our recent review, we provided an integrative framework for behavioral ecotoxicology using Tinbergen's four postulates (causation and mechanism, development and ontogeny, function and fitness, and evolutionary history and phylogenetic patterns). The aims of that review were: 1) to promote an integrative view and re-define the field of integrative behavioral ecotoxicology (IBET); 2) demonstrate how studying ecotoxicology can promote behavior research; and 3) identify areas of behavioral ecotoxicology that require further attention to promote the integration and growth of the field. In this presentation, we will discuss avenues for implementation of these ideas for SETAC members.

TP136 Drosophila: An emerging model for integrative behavioral ecotoxicology (IBET) and risk assessment

H. Long, Colorado State University, Pueblo / Biology; E.K. Peterson, Colorado State University, Pueblo / Communities to Build Active STEM Engagement

Current toxicological approaches (such as adverse outcome pathway models) to toxicity testing are problematic because of the role that emergent properties and external factors (e.g. population-level and ecosystem-level interactions) play in mediating ecologically-important endpoints. Therefore, methodology is needed to incorporate a more holistic approach to understanding how anthropogenic exposure to stressors decreases survival, including behavioral systems necessary for individual-level fitness and conservation. *Drosophila* has been established as a suitable model system for understanding behavior and is an emerging toxicology model. The aim of this presentation is to show that *Drosophila* is a suitable model system for an integrative framework approach (integrative behavioral ecotoxicology, or IBET) to incorporate emergent properties and answer ecologically-important behavioral questions. We will review the various methods for experimental exposure (ingestion, inhalation, dermal, and injection) in both field and laboratory populations, as well as ecologically-important behavioral endpoints that can be measured using this model. We will present representative results to show that *Drosophila* can be used as an IBET model to accomplish the following: 1) link multiple biological levels of organization and understand their role on phenotypic output, 2) test multigenerational and evolutionary implications of stressors, and 3) test multiple contaminants and stressors (e.g. pollutants and climate change) simultaneously.

Linking Science and Social Issues

TP137 An Agent-Based Model for Assessing Adoption Pathways of Photovoltaics (PV): A Case Study of Los Angeles

C. Grant, University of Wisconsin - Madison; A. Hicks, University of Wisconsin - Madison / Civil and Environmental Engineering

Diffusion of clean energy technologies, particularly rooftop PV, represents a key option in meeting energy demand and emissions reductions in the residential sector. Yet, one of the challenges in diffusion of PV is understanding the nature of the consumer decision-making process. The decision to adopt PV panels is a complex interrelation of upfront costs, payback time, financing and incentive availability, energy and cost savings, technology risk and complexity and consumer adoption attributes. Agent-based modeling (ABM) is a suitable tool to capture the convergence of these factors and dynamics of complex socio-technical systems. Previous work on ABM of consumer adoption of PV panels have examined patterns of adoption under varying market conditions and alternative policies. However, the majority have focused on financial incentives in the form of rebates. Additionally, most studies rely on generic survey data to model the decision rules and preferences of consumers. This research will attempt to develop an ABM for adoption of residential PV in Los Angeles county. It will allow for a range and mix of policy simulations intended to advance the adoption rate, such as clean energy standards, feed-in tariffs, carbon pricing and net metering. Agents will include homeowners who decide on solar adoption and third-party agents and installers who initiate contact with potential customers. Agents will be divided into groups based on adoption attributes such as ability to pay, attitude towards environmentally-friendly products, and other demographics generated through empirical survey data. This work is transformative in its ability to provide policymakers a means to rigorously evaluate and predict potential adoption pathways of residential PV. An understanding of the rate and pattern of consumer adoption of clean energy technologies is critical for forecasting energy demand and emissions, as well as for infrastructure planning and development. Previous studies have also raised concerns about the future availability of crucial metals that are embodied in current solar technology. Limited supply of these metals could potentially constrain the growth of this technology. As such, material flow analysis (MFA) will be combined with ABM to investigate the material requirements and supply risks associated with large-scale solar deployment.

TP138 An institutional perspective on toxicogenomics in human health chemical risk assessment: Lessons for ecotoxicologists

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Twenty years ago, the academic community identified the potential of technological and scientific advances in the omics fields of biology – genomics, transcriptomics, proteomics, and metabolomics – to improve human health chemical risk assessment. Some of this potential is realized today, with increased acceptance of toxicogenomics tools in human health risk assessment and recognition of appreciable advantages of these tools over conventional toxicity testing methods. Yet, the pace and scope of adoption of toxicogenomics tools and practices have not met early ambitious expectations (Vachon et al., 2017; Zaunbrecher et al., 2017). The social processes underlying the adoption of new practices have long been of interest to social scientists. In particular, researchers have looked at how "institutionalized" practices – those which are widely established, often unquestioned and, hence, relatively stable over time – change. One mechanism is "institutional entrepreneurship", which represents "the activities of actors who have an interest in particular institutional arrangements and who leverage resources to create new institutions or to transform existing ones". From this perspective, human health chemical risk assessment is a highly institutionalized field of professional practice that advocates of toxicogenomics seek to change through their entrepreneurial action. To explore the adoption of toxicogenomics tools and associated practices, we apply frameworks and concepts from this institutional perspective to the few published findings regarding the "barriers to adoption" of toxicogenomics tools that exist, as well as to primary case study data we collected and analyzed. Drawing from interviews with ecotoxicologists, regulators, and industry actors as well as documents and archival materials, we document the development, introduction and adoption – or not – of practices of using toxicogenomics tools in human health chemical risk assessment in the US and Canada. We theorize the main challenges faced by advocates of toxicogenomics as well as, if applicable, where, how and why these challenges were overcome. Noting important similarities and differences between health and ecological risk assessment, we translate these findings into lessons for ecotoxicologists seeking to transform ecological risk assessment of chemicals.

TP139 Assignment of task force resources to USFWS for data aggregation

A.R. Frank, B.D. McGaughey, K. Campasino, R.P. Kemman, Compliance Services International

The FIFRA Endangered Species Task Force (FESTF) has contributed to the US Environmental Protection Agency's (USEPA) endangered species and pesticide assessment process for nearly 20 years. Throughout this time, FESTF's efforts have focused on aggregating, improving, analyzing, and providing data related to endangered species and pesticides to the USEPA to inform their assessments. Through FESTF's various communications with the USEPA, the US Fish and Wildlife Service (US FWS), National Marine Fisheries Service, and the US Department of Agriculture, it became apparent that FESTF's aggregated biological information for listed species overlapped US FWS's species information and data collection efforts needs, pursuant to the Section 7 process, for developing Status of the Species (SOS) for the first national pesticide consultations through Registration Review. An SOS presents the biological or ecological information necessary and relevant in formulating the biological opinion and includes appropriate information on a species' life history, habitat needs, distribution, threats, and conservation needs. An SOS is required for every candidate, proposed, and listed species, and proposed and designated critical habitat potentially affected by the re-registration of a pesticide. In January of 2016, FESTF developed a Statement of Work for FESTF to aggregate data for the first national pesticide biological opinion due by December 2017. US FWS approved this Statement of Work and provided close supervision to the task force as work was undertaken. For more than a year, FESTF worked very closely with and under the direction of the US FWS to help assemble data that in turn can be used to populate a robust SOS section for the national pesticide consultations. This presentation will discuss the SOS project in detail, focusing on the collaboration between industry and government agencies that was required, and the resulting benefits to accelerating the availability of productive and useful results.

TP140 Community Monitoring of Organic Pollutants in Urban Recreational Fisheries

C. Clement, F. Bellows, C. Fernandez, S. Lopez, B. Polidoro, Arizona State University

In many urban areas, recreational fisheries are an important leisure activity while also providing a relatively inexpensive source of protein in local diets. However, urban recreational fisheries are generally not well-monitored in terms of fish consumption, nor are all urban surface waters regularly monitored for contaminants. Across metro-Phoenix, Arizona, a number of K-12 student groups, in collaboration with University and Community college students, are working together to detect and quantify contaminants in recreationally caught fishes and in more than 30 urban lakes and ponds. Over the past 4 years, varying levels of legacy and current-use contaminants (including pesticides, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, and phthalates) have been detected in recreationally-caught fishes, waters and suspended solids. Interviews conducted at the fishing sites indicate that more than 60% of local residents are consuming the fish they are catching in these urban waterways. As many Phoenix recreational fisheries lakes and ponds are located in low income and high minority neighborhoods, the results of this on-going study should inform urban fisheries management and other agencies of the need for fish consumption advisories, improved water quality management, and continued research and monitoring, in order to reduce potential risks to public health.

TP141 Incorporating the Joint Toxicity of Co-applied Pesticides Into the Ecological Risk Assessment Process

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Pesticides are frequently formulated as mixtures of active ingredients. Although traditionally ecological risk assessments (ERA) have been conducted primarily on individual active ingredients, there is an ongoing effort in many jurisdictions to more formally include assessment of mixtures. The overall goal of this project was to describe an approach for conducting ERA of jointly applied pesticides. We suggest that standard testing of formulation mixtures is not warranted due to the low probability of synergy occurring at a high enough magnitude to be measurable above experimental variability. Thus, empirical testing should focus on formulations where there is a greater likelihood of synergy due to known toxicological interactions of the pesticide class or *a priori* knowledge of synergy, such as intellectual property claims. Additionally, empirical testing should focus on species that are above levels of concern and limit testing on species where it is unlikely that synergy would significantly change the outcome of the ERA. If empirical testing is warranted, we suggest that results be compared to the concentration addition model (CA). If the empirical data deviates from the model by a factor of greater than five, then synergy is considered likely and the ERA is based on the empirical data. Otherwise, the ERA may use CA to calculate risk quotients or be based on the most toxic active ingredient. To evaluate the approach, we reviewed formulation mixtures where data were available. Only 3 of 24 mixture studies were found to deviate from CA by more than 5. The majority of the studies had a single component that dominated toxicity suggesting that the ERA for these formulations will not be meaningfully different if based on the most toxic active ingredient. Overall, this approach balances risk assessment conservatism and reduces testing that would likely not result in improvement of the ERA.

TP142 Municipal Wastewater Application to Forests: Using Participatory Science to Understand Human Exposure and Risks to Chemical Contaminants of Concern

M.L. Hedgespeth, North Carolina State University / Forestry and Environmental Resources; A.D. McEachran, USEPA / ORISE Research Participant to the National Risk Management; D. Rashash, North Carolina State University / NC Cooperative Extension; D. Shea, North Carolina State University; M. Strynar, USEPA / National Exposure Research Laboratory; J.A. Delborne, E.G. Nichols, North Carolina State University / Forestry and Environmental Resources

Forested land application sites (LAS) provide an alternative means of wastewater discharge in which treated effluent of municipal, industrial, and/or agricultural wastewater is applied to forest soils via slow-rate irrigation. The city of Jacksonville, NC, currently uses a forested LAS to treat and repurpose its municipal wastewater; however, population growth and non-existent land-expansion opportunities are driving discussions for alternative means to expand the capacity of its LAS, with the potential for wastewater application to surrounding agricultural land. Studies of such sites in other areas of the country have demonstrated that citizens often have concerns about their lack of involvement in decision-making processes and health and safety issues regarding wastewater reuse. For example, although such forested systems have been utilized for decades in the US, they have only recently been investigated in terms of their roles as a source of emerging contaminants into the environment. Our project goals therefore, are to characterize the potential human health risks for land-applied, secondary municipal wastewater onto the forested LAS in Jacksonville and to better understand community values and perceptions of alternative wastewater reuse to agricultural lands. To facilitate a participatory approach, the study is guided by a Participatory Leadership Team (PLT) consisting of multiple stakeholders who provide input on the chemical characterization at the site and subsequent risk assesment. Preliminary data from suspect-screening and targeted chemical analyses of water samples indicate the input of organic chemical contaminants at the LAS from wastewater into surface and ground water. Additionally, survey data obtained from a Community Involvement Group (CIG) is used to provide information on public perceptions and values regarding current, non-traditional wastewater use and risks, as well as regarding options for alternative water reuse in the future. Overall, the PLT and CIG will work together to formulate appropriate strategies to communicate these findings to the broader public.

TP143 National and Regional Environmental Injustice Trends of Waste to Energy Plants: Dioxin Emissions and Local Passive Sampling

G. Flavetta, L. Fernandez, Northeastern University / Civil and Environmental Engineering

Municipal waste to energy plants offer a more environmentally friendly solution to trash reduction than placing trash into a landfill. While many controls are implemented to keep the surrounding population safe from pollutants produced by burning trash, plants are not completely free of risk and still emit low concentrations of dioxins into the atmosphere. Measureable amounts of selected dioxins were found using passive samplers that monitored environmental concentrations near a mid-sized waste to energy plant, which currently meets federal and state emission standards. Looking at a more widespread scale, plants are often located neighborhoods with surrounding populations from lower socioeconomic classes and racial minorities. Of the 84 waste to energy plants in the United States, the larger of the plants are located disproportionally in less affluent areas and primarily minority neighborhoods. Looking at census data overlain onto national dioxin concentration modeling, we observed how race and socioeconomics factor into the location of municipal trash to energy plants and how the cite locations have changed over time.

TP144 The effect of psychological distance frames on motivation toward environmental action

E. Amel, University of St. Thomas / Psychology; C.M. Manning, Macalester College / Environmental Studies

Decades of research demonstrates that telling citizens about an environmental problem and things they can do to address it are not enough to elicit action. There are many additional psychological barriers to human behavior change (e.g., habits, social pressure, etc.). While it is intuitive to attempt to inspire behavior through incentives, long-term motivation requires intrinsic, rather than extrinsic, motivation. An action is intrinsically motivational when it fulfills basic human needs including the need for competence, autonomy and belonging. The question is what kinds of messages could increase peoples' sense of need fulfillment? To find out, we surveyed attendees at a Midwestern state fair in 2016. Embedded within the survey was a 2x3 manipulation of goal specificity and psychological distance. We presented climate change scenarios that were local or distant or had no specified location, and paired them with solutions that were either general or specific. After reading the scenario, participants responded to standard goal commitment, efficacy, and self-determination scales as well as global statements such as, "I feel like I can be a part of the solution", using a 7-point Likert scale. We found partial empirical support for the impact of psychological distance through a significant main effect of the distance manipulation on feelings of autonomy $F(2, 414)=9.54$, $p<.05$, and competence, $F(2, 414)=5.37$, $p<.05$. Specifically, people were significantly more likely to feel they possess autonomy and competence when climate change was described in terms of a specific, known place (Arizona or Minnesota) rather than an unspecified place. Thus, goals of any kind may be less motivational when people feel psychologically distant from a problem. We believe this pattern is robust to other complex environmental problems. Follow up replication data from 2017 will be presented. Implications for communication of scientific findings to the public as well as policy development will be discussed.

TP145 The Green Chemistry Initiative at the University of Toronto

S. Joudan, University of Toronto / Chemistry

The Green Chemistry Initiative (GCI) is a student-run group based out of the Department of Chemistry at the University of Toronto in Toronto, Canada. Founded in 2012, the GCI strives to promote sustainability and green chemistry practices in both the department and the broader chemical community. GCI is comprised of a wide range of students who study synthetic chemistry, catalysis, environmental chemistry and engineering. The GCI provides green chemistry information and educational resources to students, faculty and staff through a variety of initiatives, including a monthly seminar series, an annual workshop/symposium, weekly trivia questions, and online resources. The group also strives to reduce chemical waste production in the Department of Chemistry through waste monitoring, as well as waste diversion with the introduction of green bins into the department for organic food waste disposal. Other initiatives include: undergraduate curriculum development in conjunction with faculty to emphasize green chemistry concepts; a YouTube video series to explore the 12 principles of green chemistry using common reactions and analogies; a campaign to reduce energy consumption from fumehoods in the department; and most recently, a workshop highlighting the environmental fate of contaminant chemicals. This presentation will highlight the GCI's various initiatives and provide insight into the many ways that students can help reduce waste and promote sustainable practices within their own department or university campus. The role that environmental scientists play in the green chemistry community will also be discussed.

TP146 What role for toxicogenomics in the governance of chemical risk? Potential pathways to regulatory innovation in North America

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Context The roles and responsibilities of government are becoming more complex as the need to balance the demands of domestic citizenry and the global economy increases (Dhliwayo, 2017). Chemical risk assessment presents a particularly complex public policy challenge, with local to global dimensions, requiring considerable interdisciplinary and inter-sectoral dialog and broadly accepted strategies for validating and integrating new technologies into risk management frameworks (Rowlands et al., 2014). Toxicogenomics – and alternative testing technologies more generally – have been identified as having the potential to introduce important changes in the regulatory governance of chemical risks to human health and the environment. In North America, these technologies have been discussed for more than a decade as a potential answer to the chemical risk assessment challenges facing regulators. However, the accepted regulatory approaches to managing risk and the associated toolkits for prioritizing chemicals and determining their ecological toxicity has remained, for most part, unaffected. Importantly, incorporating toxicogenomic technologies in the regulatory toolkits of government raises a complex web of interdependencies involving cross-jurisdiction regulatory dialogues, large-scale research collaborations, and innovation in both technological tools and in regulatory science. As a result, core questions related to the potential for government agencies to enhance public value through innovation in domestic chemical risk assessment and regulation remain open. In particular, the barriers to, and opportunities for, regulatory innovation in public chemical risk assessment would benefit from further research to inform public policy options. Question and object: What are the challenges to, and opportunities for, toxicogenomics-driven regulatory innovation in the governance of chemical risks? Methods: Research methods will include document and policy analysis, participant observation, focus group discussions, key informant interviews and survey analysis. Aim: We will trace potential pathways for, and 'bottlenecks' affecting, regulatory innovation systems in Canada and the US, taking into account the important role played by the OECD. We aim to clarify the nature and implications of regulatory institutions in chemical risk assessment focussing on political, procedural and technical issues. This study is part of the EcoToxChip project (@ecotoxchip).

Regulatory Directions

TP147 An Overview of the EU's Biocidal Products Regulation Guidance on Environmental Risk Assessment

W.E. Hillwalker, S.D. Walker, S.C. Johnson & Son, Inc. / GSARA; P. Mason, SC Johnson EurAFNE Limited / GSARA

Global manufacturers of consumer products must meet diverse regulatory requirements before approval for placing on the market. Because pesticide substances are designed to exert an efficacious dose to target organisms while minimizing potential effects to non-target organisms, one of the key purposes of regulation is to ensure environmental safety. In the European Union, biocidal products are regulated under the Biocidal Products Regulation (BPR). To facilitate environmental assessment, the European Chemicals Agency (ECHA) have overseen the development of guidance for the purposes of predicting exposure, evaluating effects and determining risk. The framework and principles are described in the document Volume IV Environment Part B Risk Assessment, which is supplemented by a number of Emission Scenario Documents (ESD). This poster will provide an overview of key features of the environmental risk assessment framework and highlighting recent technical guidance developments. The pyrethroid insecticide chemical class, currently undergoing registration review in the United States and in Europe, will serve as an interesting case study.

TP148 Evaluating the GHS summation method in classifying acute aquatic toxicity hazard of industrial products potentially discharged in wastewater effluent

O. Kinsky, K. D'Aloia, Ecolab; T. Tran, Pace Analytical Services, Inc.; M. Osorio, N. Pechacek, Ecolab

The Globally Harmonized System of Classification and Labelling (GHS) represents an international approach to standardize classification and communication of hazards. Many industrial products are disposed with wastewater effluent. For comprehensive product stewardship, such products are assessed and appropriately classified per GHS for aquatic toxicity. For this study, commercial products used in multiple industries were initially classified for acute aquatic toxicity based on the summation method (SM) as prescribed by GHS table 4.1.3. SM is an additive approach using individual ingredient hazard categories relative to appropriate percentage thresholds to classify the full mixture. Products selected for aquatic testing were high-volume products, those with known direct effluent release into aquatic environments, or those with a challenged SM categorization. The tests used to challenge the default SM categorization were a 96-hour acute toxicity static assay conducted in freshwater species with fathead minnow (*Pimephales promelas*) or a 48-hour acute toxicity assay in aquatic invertebrates (*Daphnia magna*). Testing was conducted following methodologies detailed in the 2002 USEPA document EPA-821-R-02-012 at contract research organizations. From these standard assays, fish 96-hour LC50 and *Daphnia magna* 48-hour EC50 values were estimated and compared to GHS classification criteria to assign a test-based categorization. Of the 124 products evaluated, 57 aligned with default SM-based categorization within the acute aquatic toxicity hazard classification. For the remaining 67 products, only seven products had a test-based category elevated in hazard from the SM-based categorization, while 60 were a downgrade in hazard category. Based on this particular dataset of 124 products, the results indicate the design of the SM is working as intended by GHS, as categorization of mixtures tends to either be accurate or over-predict hazard. Category under-prediction (false negative) within this set of products was limited to a rate of 5.6% (7/124). Importantly, among the false negatives, none of these seven products differed by more than a single step in hazard category (e.g. Category 3 to Category 2). The false negative rate on categorization for acute aquatic toxicity hazard provides increased confidence that for products where testing does not exist, appropriately conservative categorization is generally provided by SM in the vast majority (94%) of products.

TP149 Lead Cleanups at Superfund Sites

C. Julias, CDM Smith

Office of Solid Waste and Emergency Response (OSWER) Directive 9355.4-12 issued a Soil Guidance in 1994 and a clarification to the guidance in 1998 for cleaning up lead-contaminated soil that present unacceptable human health and/or ecological risks from lead concentrations exceeding lead screening levels (SLs). The SL and the subsequent preliminary remediation goal (PRG) and cleanup level are calculated using the Adult Lead Methodology (ALM) and the Integrated Exposure Uptake Biokinetic (IEUBK) models for residential and non-residential scenarios, respectively, at Superfund sites. The United States Environmental Protection Agency's (EPA) Office of Land and Emergency Management (OLEM) released a memorandum on December 22, 2016 titled "Updated Scientific Considerations for Lead in Soil Cleanups," which highlights recommendations for implementing the soil lead guidance. The recommendations include basis for deriving SLs, PRGs, and cleanup levels; variation of default parameters to the ALM and IEUBK models; and role of natural or anthropogenic background levels. In addition to the memorandum, there are three other directives released on August 2, 2016 that provide recommendations for parameters used in the ALM and IEUBK models based on current scientific literature and national public health recommendations. The previous default parameters and current recommended parameters used to calculate SLs, PRGs, and cleanup levels in the ALM and IEUBK models are presented. In addition, the impact of background levels on these values is presented to provide side-by-side comparison of final lead cleanup values recommendation.

TP150 Options for derivation of site-specific selenium standards in Colorado

D. Guth, GEI Consultants, Inc. / Ecology; S. Skigen, S. Pargee, S.P. Canton, GEI Consultants, Inc. / Ecological Division

In June 2016, the USEPA released the final *Aquatic Life Ambient Water quality Criterion for Selenium*. In addition to the updated chronic water column criteria, the 2016 selenium criteria document included criteria for fish tissue, consisting of egg/ovary, muscle, and whole body elements. Due to the bioaccumulative properties of selenium, the EPA set the fish tissue criteria to take precedence over the water column criteria. Additionally, the EPA prioritized the egg/ovary element over the whole body or muscle criteria due to the tendency of selenium to accumulate in the reproductive tissues. The guidance document provides two modeling approaches to develop a site-specific water column criterion element based on fish tissue concentrations, use of a mechanistic model or use of bioaccumulation factors. While we agree with the concept of tracking each step in the bioaccumulative process for selenium, we have concerns with the variability and validation of model results, especially when using site-specific data. Furthermore, the dramatic fluctuations in flows resulting from seasonal snowmelt, combined with naturally elevated selenium concentrations throughout Colorado, appear to complicate the model usage as it was originally intended. In this presentation we provide one potential solution: using measured data to directly calculate a protective standard.

TP151 Recommendations for sieving soil and dust samples at Superfund sites for assessment of incidental ingestion via dermal adherence

M. Stifelman, USEPA / Region 10; J. Brown, USEPA / NCEA; Y. Lowney, Alloy, LLC / Health Sciences; M. Follansbee, G. Diamond, SRC, Inc. / Environmental Health Assessment; M. Burgess, USEPA / OSRTI

Incidental ingestion is the primary pathway for exposure to lead, and other contaminants, in soil and dust and is dependent on dermal adherence. Hence, site-specific risk assessment requires that soil and dust samples be sieved to accurately represent incidentally ingested material that adheres to skin. Soil and dust particle size, an important determinant of dermal adherence, is generally inversely associated with lead concentration, mobility, and bioavailability. Reliable data on the particle size fraction that is most likely to adhere to hands and on the lead concentration found in that particle size can improve the accuracy of exposure and risk calculations in lead risk assessments. We reviewed literature for relevant data on the relationship between particle size and dermal adherence, and between particle size and lead enrichment. The review revealed that growing body of evidence showing that dermally-adhered soil and dust is dominated (>90%) by particles < 150 µm. Additionally, although dependent on site-specific conditions, the review also revealed the consistent enrichment of lead associated with smaller sized particles < 150 µm. Based on this new information, USEPA now recommends that the concentration of lead in the < 150 µm fraction be used to represent the particle size fraction associated with incidental ingestion, and this particle size fraction has also been suggested for use in assessing exposure to other contaminants in soil. Previously, USEPA's Office of Land and Emergency Management recommended that the lead concentration in the < 250 µm particle size fraction be used to represent the fraction of soil and dust that adheres to hands and could be incidentally ingested. The Office of Land and Emergency Management recognizes, however, that this recommendation to sieve to 150 µm to obtain the fine fraction may be adjusted on a site-specific basis to obtain smaller or larger particle size fractions as site-specific history or circumstances warrant. For example, larger particle size fractions may be appropriate when contact with wet soil conditions are anticipated (e.g., exposures to soils or sediments at shorelines). We will provide a summary of the technical studies and regulatory analyses that form the basis for selection of this particle size range as the appropriate cutoff for use in the human health risk assessment of ingestion exposures to contaminants in soil.

TP152 Review of USEPA OCSPP Aquatic Test Guidelines

A. Samel, DuPont; M. McCoole, Bayer CropScience / Environmental Toxicology and Risk Assessment; T.M. Blickley, Dow Agrosciences / ACES; M.J. Bradley, Smithers Viscient / Sediment Toxicology; M.A. Cafarella, Waterborne Environmental, Inc. / Ecotoxicology; K.S. Henry, NovaSource/Tessenderlo Kerley, Inc.; A.J. Jones, DuPont Crop Protection / Institute of Environmental Toxicology; F. Kee, FMC Corporation; H.O. Krueger, EAG Laboratories / Aquatic, Plant and Insect Toxicology; T.S. Kung, FMC Corporation / Global Regulatory Sciences / Biochemistry and Microbiology; A. Maldonado, University of California, Riverside / Environmental Science; T. Scown, DuPont Crop Protection; T.A. Springer, Wildlife International, Ltd. / Special Projects and Technical Support; J.P. Staveley, Exponent; T.W. Valenti, Syngenta Crop Protection, LLC / Environmental Safety; J. Wang, BASF Corporation / Environmental Toxicology

In December of 2016, the USEPA Office of Chemical Safety and Pollution Prevention (OCSPP) released the final ecological effects test guidelines for select aquatic and sediment-dwelling organisms. These guidelines are for use in testing pesticides and chemical substances to develop data for submission to USEPA under the Toxic Substances Control Act (TSCA), the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), and section 408 of the Federal Food, Drug and Cosmetic Act (FFDCA), and as such are designed to be harmonized across these US regulatory programs. The test guidelines were originally released as drafts in 1996 by the USEPA Office of Prevention, Pesticides and Toxic Substances (OPPTS); these draft guidelines have been in use since then. Crop Life America (CLA) conducted a critical review of the OCSPP final test guidelines with 3 objectives. The first objective was to compare the contents of the final test guidelines with the draft versions and identify any differences; differences were not unexpected given the passage of 20 years between the release of the draft and final guidelines and the ensuing scientific advancements in ecotoxicology. The second objective was to assess the level of harmonization with, and divergence from current and comparable OECD test guidelines and assess the impact on study design for a globally accepted study. The third area of review was to identify technical aspects of the test guidelines that need further discussion or refinement. This poster highlights some of the findings, which will be discussed in detail in a future white paper, including recommendations to ensure that the results of the laboratory studies will be as scientifically and technically robust as possible.

TP153 Use of Selenium Fish Tissue Data for Reasonable Potential Analysis in NPDES Permits

S. Pargee, S.P. Canton, S. Skigen, GEI Consultants, Inc. / Ecological Division; D. Guth, GEI Consultants, Inc. / Ecology

EPA's 2016 Draft document *Frequently Asked Questions (FAQs): Implementing WQS that Include Elements Similar or Identical to EPA's 2016 Selenium Criterion in Clean Water Act Section 402 NPDES Programs* provides recommendations for conducting reasonable potential (RP) analysis for determining the need for Water Quality Based Effluent Limits (WQBELs) in NPDES permits based on the four-part selenium criterion. While the document states that any of the elements of the criterion can be used by the permitting authority to determine RP the included flow chart ultimately results in RP determination based only on water column concentrations even if fish tissue are meeting the criterion in the receiving stream. The EPA's 2016 selenium criteria document explicitly states that the tissue elements supersede the water column element. This is more environmentally relevant given that the tissue elements are based directly on measured tissue concentrations associated with toxic effects in fish, while the water column element is based on modeling and includes multiple levels of uncertainty. Because the EPA intends for tissue-based elements to supersede water column elements, this should apply in all situations, including RP analyses. We will provide examples of case studies in which fish tissues are meeting the selenium criterion and fish populations are healthy, yet selenium concentrations exceed the water column criterion. These exceedances of water column selenium concentrations would result in determination of RP for dischargers and the need for WQBELs for selenium, even though the stream is attaining the tissue-based criterion. We will also provide recommendations for conducting RP analysis using fish tissue data from systems in steady-state that are attaining the aquatic life use.

TP154 Development of a Vertebrate Toxic Agent Risk Assessment

A. Soeter, New Zealand Environmental Protection Authority / Hazardous Substances and New Organisms; R. Mohan, Environmental Protection Authority; G. Holmes, Environmental Protection Authority / Hazardous Substances and New Organisms

The New Zealand Environmental Protection Authority (EPA) is responsible for the evaluation of the health and environmental effects of hazardous substances imported into and manufactured in New Zealand. During this process, risks concerning the use of the substances are determined, and risked are weighed against the potential benefits. For a substance to be approved for use, the benefits must outweigh the risks. The native flora and fauna of New Zealand are unique due to the long isolation of the islands. The biota has developed without pressure from mammalian predators, and this has resulted in a lack of defence mechanisms. For example, New Zealand has a number of bird species which are flightless or poor flyers and nest on the ground. With the colonisation of New Zealand, mammalian predators were introduced (e.g. cats, stoats, ferrets, rats, possums). The introduction of these mammals has had such a dramatic effect on the native flora and fauna that these are now considered pest species. Several species are also considered pests in domestic and industrial settings and contribute to decreased primary production. New Zealand is therefore challenged with the control of invasive mammal pests. Several techniques are employed to control these pest populations which include trapping and shooting, as well as poisoning using Vertebrate Toxic Agents (VTAs). The poisoning of mammals is controversial, and follows two typical use-patterns in New Zealand; via bait stations and via aerial drops of the bait. The latter is applied on terrain where other methods are not possible or are too expensive. The use of VTAs is an important part of our national pest control programs. No models are readily available to estimate the risks of VTAs to the environment so the EPA has developed a qualitative and quantitative methodology to assess their potential impact. The methodology estimates the risk for all environmental factors, with special emphasis on non-target vertebrates. Birds and companion animals are considered using the most up to date allometric equations.

TP155 The Quest for Safer Consumer Products through Alternatives Analysis

D. Phelps, California Department of Toxic Substances Control

The California Safer Consumer Products regulations, created a regulatory framework to identify and prioritize consumer products as Priority Products based on their potential to contribute to adverse public health and environmental impacts. The regulations also require an Alternatives Analysis be conducted on Priority Products to evaluate alternatives and determine how best to limit exposure to or reduce the level of hazard posed by a Chemical of Concern. The AA required, parallels other lifecycle assessment tools, is divided into two stages and is a systematic process to evaluate the life cycle impacts of a Priority Product and any alternatives considered. The AA process requires that responsible entities identify factors relevant for comparison—factors that materially contribute to adverse impacts associated with the Priority product or alternatives being conserved. Based on the results of the AA a number of steps can be taken, including removal of the Chemicals of Concern, posting product information for consumers, further Research & and Development and safety measures. Ms. Phelps will provide an overview of the chemical and priority product prioritization process; duty to comply, the Alternatives Analysis process and the regulatory responses. The overview will include a focus on relevant factors to consider during the identification, screening, comparison of alternatives.

Human Health

TP156 AHR signaling and inflammation profiles in human glioblastoma multiforme cell lines differing in responses to novel experimental AHR ligands

H.R. Houke, H.R. Houke, C.D. Rice, Clemson University / Biological Sciences

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that binds natural, endogenous indole ligands (e.g., indole-3-carbinol from cruciferous vegetables), as well as select environmental xenobiotic toxicants (planar PAHs, PCBs, dioxins). The strength of ligand affinity for the AHR, and the rate of metabolic clearance of the xenobiotic, determines whether its effects lead to toxicity or beneficial outcomes such as balanced immune function and cell cycle control. We determined the effects of two indirubin (indole family) derivatives: Indirubin-3'-(2,3 dihydroxypropyl)-oximether (E804) and 7-Bromoindirubin-3'-oxime (7BIO) on AHR activity and inflammation profiles in LN-18 glioma cells and T98G glioma cells. CYP1B1 gene expression, a marker for AHR activation, is induced in both cell lines, most potently by E804. Inflammation profiles were determined using a commercial cancer-inflammation qPCR array. While CYP1B1 expression is induced by E804, expression profiles of genes indicative of pro-inflammation were highly suppressed by E804, with 7BIO having little effect. This suppression of pro-inflammatory genes by E804 was noted only noted in LN18 cells. Of note, 7BIO reduced the expression of IDO1, a key enzyme important to glioma survival, in LN-18 cells, while this compound increased expression in T98G cells. Moreover, expression of the regulatory transcription factor STAT3 was reduced by E804, but not by 7BIO in both cell lines. Thus, E804 is both an AHR ligand and inhibitor of STAT3. This work is important because gliomas (brain tumors of glial cell origin) are difficult to treat using standard modalities, and they typically suppress immune functions, both systemically and in the local tumor microenvironment. Gliomas also express phase I, II, and III components of xenobiotic metabolism, and thus are useful lines for environmental toxicology, including immunotoxicology. These two glioma cell lines differing in AHR activity levels, though connected with the same cancer type, offer the opportunity to explore the toxicity of a variety of AHR ligands on cell signaling and tumor-related inflammation.

TP157 Confirmation of high-throughput screening data and novel mechanistic insights into VDR-xenobiotic interactions by orthogonal assays

S. Kullman, D. Mahapatra, North Carolina State University / Biological Sciences

Vitamin D is a steroid hormone traditionally associated with mineral ion homeostasis. Accumulating evidence however suggest a wider biological role for vitamin D and its importance in immune function, xenobiotic metabolism, cell differentiation, neural development and neurodegeneration. Vitamin D deficiency in humans is now recognized as a pandemic and is linked to various health outcomes including neurodevelopmental deficits and obesity. Epidemiological studies have identified at risk populations for low vitamin D levels and have established putative associations with congenital birth outcomes including early gestational age, adolescent diagnosis of ADHD, and adult degenerative aberrations including Parkinson's and obesity. In this study, we utilized Quantitative high-throughput (qHTS) testing data to identify compounds that modulate vitamin D receptor (VDR) in-vitro. We demonstrate 21 potential VDR agonists and 19 VDR antagonists from a subset of >400 compounds with putative VDR activity generated by Tox21 qHTS library and followed up with additional orthogonal assays. Transient transactivation assay (TT) using a human VDR plasmid and Cyp241A1 luciferase reporter construct in Hek 293T cells revealed 20/21 active VDR agonists and 18/19 active VDR antagonists. Compounds were then examined using mammalian-2-hybrid assay (M2H) to evaluate VDR interactions with co-activators and co-regulators. With the exception of a select few compounds, VDR agonists exhibited minimal to moderate recruitment of co-regulators and co-activators whereas antagonists exhibited moderate to marked attenuation of co-activator recruitment by vdr both in the presence and absence of co-regulators. A unique set of compounds exhibiting synergistic activity in antagonist mode and no activity in agonist mode was identified. Additionally, bioinformatic clustering and heat map and cheminformatic modeling of VDR-ligand interactions were performed. Overall, data emphasizes the molecular complexity of VDR transcription machinery in terms of differential and preferential affinities of compounds for co-regulators/co-activators and respective sites of action. Further research will help decipher pathways that these chemicals follow to attain functional outcomes.

TP158 Exposure to Air Particulate Matter: Is Indoor Environment a Safe Heaven?

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Urban residents spend the majority of time in indoor environments, which, however, may not be a safe haven for staying away from outdoor air pollution. To assess the indoor air exposure, the particle mass concentration (size with 0.056-18 μm) and number concentrations (size with 14-660 nm) as indices were simultaneously measured inside and outside of three typical urban settings, i.e., school, office and residence in Guangzhou, China from October-November 2014 (dry weather season) and June-August 2015 (wet weather season). The total particle mass concentrations were significantly higher in the dry weather season than in the wet weather season ($p < 0.05$), but the fine particle number concentrations were not seasonally variable for all sampling sites, suggesting the size dependence of particle scavenging efficiency by wet deposition. It is interesting to note that the particle number concentrations of indoor were positively correlated with outdoors at schools and offices ($r^2 = 0.91-0.95$, $p < 0.001$). Furthermore, the infiltration factors (F_{inf}) and indoor/outdoor ratio (I/O ratio) of particles were 0.48-1.03 and 0.81-1.05. Even in haze episode, the average I/O ratio of fine particles hits 0.82. All findings suggested an efficient exchange between indoors and outdoors, which confirmed the hypothesis that indoor environment is not a safe shelter to avoid air pollution.

TP159 Profile of indoor air in a university in Nigeria

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The indoor air quality of some selected sites at Federal University of Technology, Owerri was analyzed using the Buck Air Sampler. The indoor air was sampled during the dry and wet seasons as well as in the morning and afternoon periods. Microbiological analysis of total heterotrophic bacterial counts and total heterotrophic fungal counts were recorded. Results from the study revealed that there were higher bacterial counts (202.3 CFU/M^3) and fungal counts (23.3 CFU/M^3) in dry season than in the wet season. The microorganisms isolated were *Bacillus* sp, *Micrococcus* sp, *Staphylococcus* sp, *Klebsiella* sp, Streptococcussp, Fusariumsp, *Penicillium* sp, *Aspergillus* sp and *Mucor* sp.The highest bacterial count was recorded in the afternoon during both seasons indicative of the influx of students and others at that time of the day. *Staphylococcus aureus* was the most frequently detected bacterium (100%) whereas *Penicillium* sp (71.4%) was the predominant fungus isolated.The microbial concentration of indoor air observed was above the range established by WHO. The presence of pathogenic airborne microorganisms observed in the indoor air was of great concern as these pathogens play significant roles in causing diseases and disorders in humans.

Environmental or Analytical Chemistry – Part 2

TP160 An alternate configuration of the organic-diffusive gradients in thin films passive sampler for polar organics in aquatic systems

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Previous literature has shown the utility of the diffusive gradients in thin film sampler for polar organics (o-DGT) as an effective passive sampler in aquatic environments. In this study, a new configuration with a polyacrylamide diffusive gel and SepraÔ ZT sorbent (Phenomenex$^{\grave{O}}$) was developed and calibrated at multiple pH values. Linear uptake ($r^2 > 0.9$) was observed at pH»5 for a suite of 31 pharmaceuticals and pesticides over 25 days; suitable for typical passive sampler deployments. The same experiment was performed at pH»8.5 which resulted in linear uptake ($r^2 > 0.9$) for many of the same compounds. Calculated sampling rates for both experiments ranged from 3.5 (paroxetine, pH=8.5) to 14.8 mL/d (enrofloxacin, pH=5). Comparisons of the uptake rates between the two pH experiments generally agreed (10% average relative error), with only six compounds exhibiting marked reduction with pH (e.g. sulfonamide antibiotics). Sampling rates with polyacrylamide gel were slightly lower than that observed previously for o-DGT with agarose, given more restrictive pore sizes in the former. Samplers performed well in field evaluations conducted in a wastewater treatment plant and an impacted river system. This work suggests that the introduction of an outer polyacrylamide diffusive gel and SepraÔ-ZT binding gel makes for a more resistant and cost effective sampler, compared to earlier designs.

TP161 comparative analysis on methods of extractions of lead in decorative paint around lagos

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Potentially toxic metals (PTMs) are metals of high density that are relatively toxic in the environment even at low concentration. Lead as potentially toxic metal affects the human body system when it finds its way into it,especially in infants. Lead when absorbed hinders the formation of heamoglobin hence causes a lot of other health disorderliness in humans. The aim of this project work is to access the level of lead in decorative paints on walls of houses in Lagos Nigeria. Twelve paint samples were collected from walls of different buildings around Lagos, digested and analysed using Flame Atomic Spectrophotometer (FAAS). Lead from the paint samples were extracted using three different methods. A mixture of HNO_3 and $HClO_4$, a mixture of HNO_3 and H_2O_2, dry ashing in a muffle furnance followed by leaching with HNO_3. The analysis were done using the flame absorption spectrophotometer (FAAS). There was no significant difference between the methods of extraction when compared with ANOVA at 95% confidence level.This shows that all the three methods are good for extraction of lead in paint chips. Although extraction with HNO_3 and $HClO_4$ showed highest recovery of 81.93% while that of dry ashing showed the lowest recovery of 66.70%. It can be conclusively stated that all the paint samples contains significant amount of lead,although some exceeds the USEPA limit of 90PPM.

TP162 Determination of Glyphosate and correlated in Environmental Water Samples by Liquid Chromatography Coupled to Orbitrap Mass Spectrometry

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Glyphosate is a common broad-spectrum systemic herbicide used widely to kill weeds especially annual broadleaf weeds and grasses known to compete with crops. Usually Glyphosate, as it is very polar, undergoes FMOC derivatization by reacting the native glyphosate with fluorenylmethyloxycarbonyl chloride (FMOC-Cl) before analysis. This derivatization step complicates the analysis and there is a growing need for a method which can detect not only Glyphosate (and it´s major metabolite AMPA) but also Glufosinate and similar highly polar compounds, in their underivatized states. Additionally, an important loss in sensitivity can occur due to a high degree of interference introduced during the derivatization procedure. Therefore, the use of lengthy cleanup procedures is often required to achieve satisfactory analytical precision and accuracy for glyphosate and AMPA A novel method based on high performance mixed mode liquid chromatography coupled to Orbitrap mass spectrometry (HPLC-Orbiytrap/MS) was developed for the sensitive determination of glyphosate, its major degradation product, AMPA and glyphosinate in environmental water samples. The method involves the use of MS compatible mobile phases (ammonium formate 50 mM and acetonitrile) for HPLC and direct analysis of water samples without sample derivatization. The method has been validated followed the EPA guidelines by accuracy and precision studies All mean accuracy values ranged from 80% to 115% for glyphosate and AMPA with a mass tolerance window set to ± 3 ppm mass error and 60K of resolution power.

TP163 Determination of Perfluoroalkyl Substances, Phthalate Esters, Nonylphenol, Bisphenol A, Gallium, and Indium in Foods and Water

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Perfluoroalkyl substances (PFASs), phthalate esters (PAEs), nonylphenol (NP), and bisphenol A (BPA) are emerging contaminants and are ubiquitous in the environment. These compounds are widely used in many consumer and industrial products such as food container, plastics, personal care products, and surfactants. Gallium (Ga) and Indium (In) are two common metals in high-tech industries, such as semi-conductors and liquid crystal panels. They may have adverse effects on reproduction and development and may disrupt endocrine systems. Consumption of contaminated water and food is the major exposure route. This study developed a method of determining ten perfluoroalkyl substances (PFBA, PFPeA, PFHxA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFHxs, and PFOS), six phthalate esters (DEP, BBP, DEHP, DNOP, DINP, and DIDP), NP, BPA, Ga, and In in six types of foods including pork, pork liver, pork kidney, fish, clams, and oyster using QuEChERS sample preparation, and in water with solid-phase extraction (SPE), and microwave assisted acid digestion on metal analysis. Organic and inorganic detection were with UPLC-MS/MS and ICP-MS, respectively. PAEs were separated on an Ascentis Express F5 column (30 × 2.1 mm, 2.0 µm) with mobile phases composed of (B) methanol and (A) 5 mM ammonium acetate$_{(aq)}$ (pH = 6.56) at ESI+. PFASs, NP, and BPA were separated with a BEH C18 column (50 × 2.1 mm, 1.7 µm) with mobile phases composed of (B) methanol and (A) 10-mM *N*-methylmorpholine$_{(aq)}$ at ESI-. Acidification of the aqueous phase and use of dispersant methanol at QuEChERS did not increase the extraction efficiencies. Besides, Enhanced Matrix Removal-Lipid (EMR-Lipid) was suitable for the cleanup of dSPE because it could remove lipids in food matrixes without losing analytes. The matrix effect factors of pork, pork liver, pork kidney, fish, clams and oyster were 63.6-168%, 58.0-147%, 63.7-153%, 60.9-198%, 63.5-149% and 50.0-153%, respectively. The extraction efficiencies on spiked pork, pork liver, pork kidney, fish, clams and oyster ranged from 49.9-96.8%, 50.9-95.7%, 49-93.7%, 49.7-104%, 38.5-105%, and 53.3-106%, respectively. Recoveries of gallium and indium at three different spiked levels in pork, pig livers, pig kidneys, fish, and oysters were both about 85%. Standard Reference Material 2782 (industrial sludge) was used for method validation, and both gallium and indium were within the range of the certified concentrations. The the method will be applied to real samples.

TP164 Determination of personal care products and pharmaceuticals in river sediments, KwaZulu-Natal, South Africa

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A quantitative method is described for ultrasonic-assisted solid-phase extraction (SPE) followed by GC-MS after derivatization for the simultaneous analysis of personal care products and pharmaceuticals (PCPPs); propyl paraben, triclosan, caffeine, carbamazepine and chloramphenicol. Ultrasonic combined with centrifuge were used to extracts sediments samples collected from Mgeni and Msunduzi River. An SPE procedure was used for clean-up and to concentrate selected compounds from diluted aqueous extracts. Final extracts were derivatized with BSTFA and analysed with GC-MS in selected ion monitoring (SIM) mode. The recoveries of the analytes ranged from 66% to 108%. The method detection limits were 0.08 – 1.82 ng g^{-1} and quantification limits 0.42 – 5.51 ng g^{-1}. The proposed method was applied in the evaluation of two rivers over the three-month period in KwaZulu-Natal, South Africa. All targeted compounds were present in the environment at concentration level between not detected to 174 ng g^{-1}. To our knowledge, this is first reported on the simultaneous determination these PCPPs by GC-MS in Africa.

TP165 Discovery of a widespread metabolic pathway within and among phenolic xenobiotics

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Metabolism is an organism's primary defense against xenobiotics, yet it also increases the production of toxic metabolites. It is generally-recognized that phenolic xenobiotics, a group of ubiquitous endocrine disruptors, undergo rapid phase II metabolism to generate more water-soluble glucuronide and sulfate conjugates as a detoxification pathway. However, the toxicological effects of the compounds invariably point to the phase I metabolic cytochrome P450 enzymes. Here we show that phenolic xenobiotics undergo an unknown metabolic pathway to form more lipophilic and bioactive products. In a nontargeted screening of the metabolites of a widely used antibacterial ingredient: triclosan (TCS), we identified a metabolic pathway via in vitro incubation with weever, quail, and human microsomes and in vivo exposure in mice, which generated a group of products: TCS-O-TCS. The lipophilic metabolite of TCS was frequently detected in urine samples from the general population, and TCS-O-TCS activated the constitutive androstane receptor with the binding activity about 7.2 times higher than that of the parent compound. The metabolic pathway was mediated mainly by phase I enzymes localized on the microsomes and widely observed in chlorinated phenols, phenols, and hydroxylated aromatics. The pathway was also present in different phenolic xenobiotics and formed groups of unknown pollutants in organisms (e.g., TCS-O-bisphenol A and TCS-O-benzo(a)pyrene), thus providing a cross-talk reaction between different phenolic pollutants during metabolic processes in organisms.

TP166 Do common XAD extraction methods used to generate extracts from disinfected water adequately link extract toxicology to disinfected water chemistry?

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Motivation: It is common to use XAD resins to extract disinfection byproducts (DBPs) from disinfected water. The resulting extract is used in toxicological assays to study the effects of DBP mixtures and has been considered representative of the original disinfected water. However, many DBPs of toxicological concern may elude capture via the resins due to volatility, incompatibility with the resins, or from the elution and concentration process. As such, the resulting extract may not be representative of the original water DBP mixture. The purpose of this study was to determine the efficacy of XAD resins for extracting DBPs. Methods: Water was collected from a drinking water treatment plant before entering disinfection tanks. The water was spiked in our laboratory with bromide and iodide, disinfected by either chlorine or chloramine, and extracted by XAD resins. The disinfected water, both before quenching with acid, and after XAD extraction, was sampled for DBP quantification. We show concentration-response data (the same source water spiked at four bromide and iodide concentrations) for eight DBP classes generated by chlorination and chloramination, totaling 51 DBPs quantified. Results: Comparing DBP concentrations in the chlorinated water going on the resin to the water leaving the resin, total organic halides (TOX) and individual haloacetic acid (HAA) analyte removal (i.e., extracted+lost) ranged from 50-64% and 0-65%, respectively. Chloramination reactions generated total organic nitrosamines (TONO) (up to 19.1 ng/L as NDMA) and with some chloraminated samples, HAAs and TONO were either generated, or better detected in the water leaving the resin (7-100% and 14-18% increase, respectively). Trihalomethanes (THMs) were removed 100% in chloraminated samples; in chlorinated samples, removal ranged from 24-100%, with most THM analytes removed >52%. In future work, we will attempt to quantify DBPs in the extracts to complete the mass balance, determining which DBPs were extracted and which were lost in the process, or from volatility. Conclusions: Depending on the disinfectant and DBP class, concentrations in sample water before and after XAD resin extraction vary widely; suggesting XAD resin extraction may not preserve DBP concentrations and ratios of the original water. To generate extracts of DBP mixtures that accurately represent their origins, the prevailing XAD extraction technique needs modification. (This abstract does not represent EPA policy.)

TP167 Effective screening of the estrogen-receptor active compounds in environmental water using bio-mimic adsorbents prepared by molecular imprinting

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Recently, the effect to ecological system by endocrine disrupters in environment is under scrutiny, so that we attach importance to the risk management of endocrine disrupting mechanism by many of chemicals. On the other hand, the test for endocrine disrupting in vivo leads high cost and long-haul, the place in the order for priority of the evaluations is usually needed. As first screening of the candidate as endocrine disrupters, although the commonly used hydrophobic adsorbent, C18 silica is usually employed, the letting slip of hydrophilic active compounds and large amount of contamination by non-specific adsorption are always become issues. To overcome the problems, we focus on the development of an "activated-compounds selective" adsorption media using an advanced molecular imprinting technique. In brief, we aim to prepare new adsorbents for selective separation of the compounds, especially for 17β-estradiol (E2), which are active for an estrogen-receptor (ER). Previously, the molecularly imprinted polymers (MIPs) for E2 have been reported, however, the hydrophobic non-specific polymer matrix was not suitable for the real screening and analysis for environmental water samples. In present study, we newly prepared the MIPs with the concepts containing a hydrophilic polymer matrix by poly(ethylene glycol) based polymer, using hydrophobic aromatic monomers like a mimic of hydrophobic pocket in proteins, and multiple hydrogen bonding by a few hydrophilic monomers. The prepared MIPs were evaluated with simple batch adsorption and liquid chromatography to confirm the presence of the selective recognition sites for ER-active compounds. Furthermore, the optimized MIPs were utilized for the screening of the compounds from real environmental water samples. As results, the MIPs effectively showed the adsorption selectivity for ER-active compounds. Additionally, the complete removal of ER-active compounds were achieved from a number of real samples without less non-specific adsorption (less than half) compared to C18. Finally, we effectively found the candidates for ER-active compounds from real samples by analyses in TOF-MS detection. We believe that our suggested concept can be useful for screening, analysis, and exploring the unknown ER-activ

TP168 Effectiveness of the Engineered Wetlands in Removing 85 Pharmaceuticals and Personal Care products in Municipal Wastewater

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Pharmaceutical and personal care products (PPCPs) as emerging environmental contaminants have raised public health concerns due to their strong endocrine disrupting activity. Several classes of PPCPs such as fluoxetine (antidepressant), erythromycin (antibiotics), iopromide (X-ray contrast), atenolol (b-blocker), and estrone (estrogen), ethinyl estradiol (treatment for menstrual and menopausal symptoms), triclosan (antibacterial and antifungal), bisphenol A (chemical in plastic), 4-nonylphenol (component of household detergents), and progesterone (endogenous steroid hormone) are persistent and frequently found in effluents from municipal and hospital wastewater treatment facilities or tile drainage following surface application of dewatered municipal bio-solids. Several endocrine disruptive PPCPs can adversely affect normal reproductive, behavioral, immune system, and neurological functions of aquatic organisms. Prior study showed that WWTP effluent was likely responsible for reproductive impairment including intersex and vitellogenin induction in fish. Engineered wetlands have been successfully used as tertiary treatment process for removal of oxygen demand (BOD), total suspended solids (TSS), organic matter, nitrogen, and phosphate from domestic wastewater. However, there is a lack of information about the effectiveness and efficiency of this low-cost, environmentally friendly tertiary treatment process in removal of the PPCPs. The objective of this study is to evaluate the effectiveness of the constructed wetlands in removing the selected 85 persistent PPCPs in the municipal wastewater. The extraction and analytical methods have been successfully developed during 2014 – 2015 to quantify the more than 80 PPCPs in the water samples collected from the wetlands. The findings of this study will help to improve the design of wetlands for removal of PPCPs.

TP169 High-Throughput Determination and Characterization of Short-, Medium-, and Long- Chain Chlorinated Paraffins in Human Blood

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The industrial chlorinated paraffins (CPs) are comprised of short-chain (SCCPs), medium chain (MCCPs), and long chain (LCCPs) CPs. Although SCCPs and MCCPs are environmentally ubiquitous, little is known about CPs in humans. This study established a method for simultaneous determination of 261 SCCP, MCCP, and LCCP congener groups in one injection by reversed ultra-high-pressure liquid chromatography coupled with chlorine-enhanced electron spray ionization-quadrupole time-of-flight mass spectrometry. The method yielded good peak shapes, high sensitivities, and low co-eluted interferences for all examined CPs. LCCPs with carbon numbers of 21 to 27 were detected in their standard technical mixtures, and MCCPs and LCCPs impurities were detected in the LCCP and MCCP standard technical mixtures, respectively, causing quantification deviations when these mixtures were used for calibration. After considering these impurities' contribution to the total concentrations, the quantification accuracies for ∑SCCPs, ∑MCCPs, and ∑LCCPs ranged from 95.1±8.4% to 105.6±9.2% in the eight CP technical mixtures. The method was successfully applied to determine CPs in about 6 g human blood samples from a general population, and estimated ∑SCCP, ∑MCCP, and ∑LCCP concentrations to be 370-35,000, 130-3200, and 22-530 ng/g lipid weight (n=50), respectively. A comparison of blood and soil/air CP profiles from the same areas suggested a relatively higher potential for the accumulation of SCCPs, compared with MCCPs, in humans.

TP170 Occurrence and exposure to phthalate metabolites in urine from Korean mother-infant pairs

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Phthalates are a group of chemicals widely used in consumer products including plasticizers, cosmetics, and personal care products (PCPs). Humans are exposed phthalates through food, dust, air, and commercial products. Due to the short half-lives of phthalates in human body, urinary phthalate metabolites have been used as biomarkers of human exposure to phthalates. Unlike adults, infants have additional exposure sources of phthalates such as baby care products, toys, and dust as well as food. Infants are vulnerable group due to low metabolic capacity and small body burden. In our study, 18 phthalate metabolites were measured in 127 mother-infant (36-54 months) pairs (*n*=254) to investigate the exposure levels and sources of phthalates.Total concentrations of phthalate metabolites ranged from 3.6 to 365 ng/mL (average ± SD; 68 ± 53 ng/mL), and 13 to 1081 ng/mL (134 ± 140 ng/mL) in urines from mothers and infants, respectively. No significant differences were found for the concentrations of phthalates according to the infant's age(36, 42, 48, and 54 months). Mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP) showed the highest concentration. Monoethyl phthalate (MEP), monobutyl phthalate (MBP), and monomethyl phthalate (MMP) showed relatively higher levels in urines, indicating the influence of exposure of dust and cosmetics. Compared to mothers, infants showed higher concentration of all of the phthalate metabolites. Significant correlations were found for DEHP metabolites, MiBP (monoisobutyl phthalate), MBzP (monobenzyl phthalate) and MeP between mother and infants. This result indicates that they have similar exposure sources for mothers and infants. Among age group, 3-year-old infants showed strong correlation ($r = 0.553$, $p < 0.01$) of total concentrations of phthalate metabolites with their mothers. With increasing age of infants, no correlations were observed for the phthalate concentrations between mothers and infants, implying extensive contamination sources of phthalates in our daily life. Further studies are required to identity the exposure sources of phathalates associated with infant's behaviors and diets.

TP171 Residual Characteristics and Risk Assessment of Diflubenzuron and Lambda-cyhalothrin in Spinach and Korean Cabbage

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This study was carried out to investigate residual characteristics and risk assessment of diflubenzuron 25% WP and lambda-cyhalothrin 1% EC in Spinach and Korean cabbage under green house conditions. The test pesticides, diflubenzuron and lambda-cyhalothrin, were diluted 2,500 times and 1,000 times, respectively and then those were sprayed 2 times at 7 days interval 0, 3, 7 and 14 days before harvest on Spinach and Korean cabbage. Samples were collected from harvest day(0 day), 3, 7 and 14 days after last spraying. Recoveries of diflubenzuron ranged from 89.1% to 97.4% in Spinach and Korean cabbage and from 89.8% to 104.8% for lambda-cyhalothrin. Residual concentration of the test pesticides in Spinach and Korean cabbage were reduced time-coursely. The percent ratios of EDI to ADI (%ADI) of the test pesticides in Spinach and Korean cabbageusing a average food daily intakes in Korea. %ADI in spinach and Korean cabbage were 9.1 and 17.5% for diflubenzuron and 0.4 and 0.6% for lambda-cyhalthrin, respectively. These results indicated that the residue levels of diflubenzuron and lambda-cyhalthrin in the test crops do not pose any unacceptable risk to human health.

TP172 Risk Assessment of Exposure using a MOS for Clothianidin Applicator on Paddy Field with Power Sprayer

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This study was carried out to assess clothianidin exposure using a margin of safety (MOS) with a whole body dosimetry (WBD) during clothianidin+flubendiamide (2+4)% SC was spryed with a power sprayer onto ten paddy fields by ten operators at a rate of 0.04 kg ai/ha. Operators put on disposable working clothes and carried the personal air pump with fiber glass filter in IOM sampler which set up flow rate at 120 L/h during application. Commercial pesticide was diluted 1,000 time and then sprayed on to field of 1 ha area. Samples were collected just after pesticide spraying. Instrumental limit of quantitation (LOQ) of the test pesticide analyzed with an LC-MS/MS was 0.001 mg/kg. Recoveries in the matrix, such as fiber glass filter, washing solution for hands and gloves, gauze for face and neck, and outer clothes, ranged from 71.3 to 113.3%. Exposure rates were calculated by comparing exposure amount to application amount of active ingredient. Total exposed amounts to parts of worker were 0.000097% for hands and gloves, 0.0011% for arms, 0.0060% for body and hip (total amount of front and back) and 0.0130% for legs of the total sprayed pesticide. Inhalation exposures with a IOM sampler on personal air pump were less than 0.00001%. Total exposures including all the body parts and inhalation were less than 0.021% comparing to total application dose. Risk assessment of exposure on applicator to clothianidin during application in paddy field with power sprayer was carried out by calculating MOS. The MOS in this experiment was 215.8, indicating a low possibility of risk because of the MOS calculated was more than 1.

TP173 Selective separation of estrogen-receptor active compounds by a bio-mimic adsorbent using an enlarged molecularly imprinted polymer

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In recent years, the screening test of endocrine disrupters in environmental water is regarded as an important task. Usually, in vivo screening is employed, which is time- and cost- consuming. Some other biological methods, e.g., enzyme-linked immunosorbent assay, are physically and chemically unstable. Therefore, a new effective screening technique is strongly required instead of present methods. In this study, we aim to achieve the preparation of highly selective molecularly imprinted polymer (MIP) toward the active compounds for an estrogen receptor (ER) as a novel separation medium for new effective screening technique. The well-designed binding sites of the MIP are expected to work as an ER mimic. Briefly, the concept includes 1) a highly hydrophilic matrix consisted of poly(ethylene glycol) diacrylate (PEGDA) polymer network, 2) a hydrophobic aromatic binding site, and 3) hydrogen-bonding provided by a variety of functional monomers. To complete the concept, a number of MIPs were prepared with PEGDA as a crosslinker, a hydrophobic monomer or benzyl methacrylate (BMA) and hydrophilic monomers, i.e., methacrylic acid (MAA), 4-vinylpyridine (4VP) and 4-imidazoleacrylic acid (IAA), as functional monomers, estriol as a template molecule, and water/*N,N*-dimethylformamide as a porogenic solvent. The adsorption selectivities of the MIPs and non-imprinted polymers (NIPs) were evaluated as follows: The polymers were immersed in an aqueous solution including 100 ppb ER active and non-ER active solutes for 1 day. Then the supernatant was measured by LC/MS/MS to determine the concentration of each free solute. As a result, the MAA and IAA based MIPs provided a highly selective adsorption ability to the ER active compounds. Finally, we demonstrated the separation of the ER active compounds from a pseudo environmental water samples with typical solid-phase extraction (SPE) using the MIPs as adsorbents. The MIP-SPE showed the higher recovery for the ER active compounds with a low interference from the background compared to conventional hydrophobic SPE. Moreover, we found that the contents from the MIP fraction included unknown ER active candidates.

TP174 Simultaneous analysis of fourteen endogenous steroid hormones by liquid chromatography mass spectrometry with atmospheric pressure photoionization

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Exposure to endocrine active chemicals can lead to perturbations of the hypothalamic-pituitary-gonadal (HPG) axis, ultimately leading to adverse reproductive or developmental effects. To evaluate potential effects, studies with possible HPG-active chemicals often rely on radioimmunoassay (RIA) for determination of biologically-active steroids and their precursors in biological matrices. While RIAs provide high sensitivity, there is potential for cross reactivity of antibodies, and assays are limited to a single steroid. To address these limitations, in the present study analytical methods were developed for the simultaneous analysis of fourteen steroid hormones including androgens, estrogens, progestogens, and glucocorticoids by liquid chromatography tandem mass spectrometry (LC-MS/MS). Atmospheric pressure photoionization (APPI) with a toluene dopant was utilized to allow ionization of all compounds in positive ionization mode without the need for derivatization. This method was applied to the analysis of fathead minnow (*Pimephales promelas*) plasma and exposure tank water from zebrafish (*Danio rerio*) experiments. Application to tank water analysis during flow-through chemical exposures provides a possible non-invasive endpoint for time-course experiments. The method demonstrated high sensitivity in both matrices with detection limits of most steroids at low µg/L in plasma and sub ng/L in water. Application of this method to aquatic vertebrate toxicity testing will lead to better understanding of specific mechanisms of HPG axis disruption and inform development of adverse outcome pathways (AOPs). The contents of this abstract neither constitute, nor necessarily reflect, official USEPA policy.

TP175 Sorption-desorption of quinclorac in an agricultural soil amended with eucalyptus, rice hull, and native bamboo biochar

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Quinclorac (3,7-dichloroquinoline-8-carboxylic acid) is a herbicide used for post-emergence weed control on grass and turf. Mobile herbicides as quinclorac are of concern for contaminating groundwater. However, biochar has been shown to be a highly effective adsorbent in soil retention of herbicides, consequently reduces the leaching of these herbicides. The objective of this research was to evaluate the sorption-desorption behavior of quinclorac, in an agricultural soil amended with eucalyptus (*Eucalyptus grandis*), rice hull (*Oryza sativa*), and native bamboo (*Merostachys skorvotzii*) biochar. The biochars were added to soil at 0 (control – unamended) and 1% (w w^{-1}) ratio, corresponding 0 and 12 t ha^{-1}, respectively. [2,3,4-^{14}C] quinclorac sorption and desorption studies were evaluated by the batch equilibrium method. The K_f value calculated for the quinclorac sorption on the unamended soil was 0.81 µmol $^{(1-1/n)}$ $L^{1/n}$ kg^{-1}. The K_f value calculated for the quinclorac desorption on the unamended and biochar-amended soil ranged from 2.21 to 3.67 µmol $^{(1-1/n)}$ $L^{1/n}$ kg^{-1}. The $1/n_{(desorption)}$ are greater than $1/n_{(sorption)}$, suggesting that the quinclorac sorption by unamended and biochar-amended soil was reversible. H values ranged from 1.19 to 1.10 for quinclorac on all treatments. Of the 3 biochars studied, native bamboo was the most efficient in the retention process of quinclorac in amended soil compared to unamended soil. Overall, eucalyptus, rice hull, and native bamboo biochar slightly increased sorption and decreased desorption of quinclorac, being these biochars a poor sorbent for this herbicide.

TP176 Spatial distribution of mercury in marine sediments from Cartagena's Bay, Colombia

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Spatial distribution patterns of total mercury (THg) in ten surficial marine sediment samples (M1-M10) around Cartagena´s Bay were evaluated. The knowledge of the distribution of trace metals in marine sediments is important because they are sinks of natural and anthropogenic substances that are preserved in sediment particles or recycled to the water column. One of the most toxic metals for the biosphere is the Hg, which has a great affinity with the particulate phase and, in contaminated coastal sediments, its concentration commonly shows the historical evolution of the contamination with this metal. Is important to identify the potential risk of Hg contamination and toxicity to the marine environment and humans. The presence of mercury in the Bay of Cartagena is due to accidental spills in the industry, and by illegal mining in the south of Bolivar and Antioquia Departments, that descends by the Magdalena River and empties by the Dique channel to the Bay of Cartagena. This study evaluated the spatial distribution of total mercury concentrations; they were analyzed in marine sediments to assess the level of contamination along of ten points from Cartagena Bay. The samples from marine sediments were analyzed by Lumex RA-915M mercury analyzer with PYRO-915+ module for pyrolysis. DORM-3 was used as the Standard reference material by the NRCC (National Research Council of Canada). Concentrations of Total Hg (THg) found in the marine sediment samples were in a range of 2,5-9,9 ng/kg dry weight for samples M1,M2,M3, M9 and M10. M5 (0,07 ng/kg), M7 (0,8 ng/kg). The majority concentrations of Hg total were found in the samples M4 (79,6 ng/kg), M6 (31,5 ng/kg) and M8 M8 (28,2 ng/kg). Concentrations of mercury total in the sediment samples were low in accordance with National Oceanic and Atmospheric Administration's for sediment toxicity to benthic communities. But, there is an approximate fraction of the 10% that is available in the ecosystem to enter the food chain.

TP177 Variation to Fluorescence Characterization of Allochthonous Dissolved Organic Carbon due to pH changes

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Dissolved organic carbon (DOC) is an operationally defined parameter that encompasses a variety of chemical structures dependent on the specific allochthonous or autochthonous carbon source. Due its speed and convenience, excitation-emission matrix (EEM) fluorescence spectroscopy has become a common technique used to characterize DOC. The type of DOC has a significant impact on both the chemical and biological processes that occur in natural waters, as well as the disinfection by-product formation during drinking water treatment. Different kinds of leaves were extracted using pH adjusted lab water, then analyzed with a fluorimeter. We investigated the changes to the DOC EEM signature associated both with the pH of the extraction water as well as the final pH of the analyzed leachate. This data furthers our knowledge about the robustness of the technique, as well as the potential pitfalls in comparing spectra from different samples.

Existing and Emerging Contaminants in Changing Arctic Environments

WP001 Increasing Mercury and other elements in Arctic Char from a lake undergoing climate-driven changes

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This study utilizes two adjacent, geologically similar lakes, West and East, at the Cape Bounty Arctic Watershed Observatory (74°55′ N, 109°35′ W) on Melville Island in the Canadian Arctic, whose catchments are currently undergoing climate-driven changes. The West catchment experienced numerous large active layer detachments during 2007-08, and the lake experienced sustained turbid conditions due to sub-aqueous slumping in 2008, and in 2011-12. East and its catchment experienced relatively minor disturbances. We hypothesize that increased erosional inputs into West Lake will result in increasing mercury (Hg) concentrations in landlocked arctic char (*Salvelinus alpinus*) and other bioaccumulative elements such as cesium (Cs) and rubidium (Rb). To investigate this arctic char have been collected annually at the end of July, from 2008 to 2016, and analysed for a suite of 34 elements using ICP-MS, and Hg using USEPA Method 7473. Carbon (C) and nitrogen (N) stable isotope analysis showed that char have significantly more depleted $\delta^{13}C$ in East vs West Lake (mean ± SD; -27.26±0.82 ‰ (N=96) vs -24.72±1.10 ‰ (N=101)) indicative of greater terrestrial carbon inputs to West Lake. Also $\delta^{15}N$ is significantly lower in West Lake char (10.1±0.99 ‰ vs 11.2±0.51‰) suggesting differences in food sources. The combined results from 2008 to 2016 collections show that the West Lake adult char have significantly higher concentrations of Hg, Cs and Rb in muscle (geomeans 152, 3.6 and 1760 ng/g wet weight, respectively) compared to East Lake (87.0, 2.1 and 890 ng/g) and this difference is even greater if results are adjusted for $\delta^{15}N$ or length. Condition factors (g*100/cm^3) for char in West Lake have declined since 2008 and over the period 2011-2016 have been significantly lower (0.60±0.11) than those in East Lake (0.67±0.08) indicating they are thinner than fish of the same length in East Lake. This may be due to difficulty feeding in West Lake's turbid waters. Hg concentrations have declined in East Lake char over the period 2008 to 2016 (averaging -5.3%/yr) while increasing (4.6%/yr) in West Lake from 2009-2016. Cs and Rb in char muscle have also increased significantly in West Lake since 2009 while showing no change in East Lake. The higher concentrations and increasing Hg, Cs and Rb in West Lake char are consistent with higher inputs into West Lake resulting from extensive permafrost disturbance in the West watershed and subaqueous slumping.

WP002 Methods for modeling the effects of climate change on contaminant temporal trends in marine, terrestrial and avian wildlife of the Canadian Arctic

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The Northern Contaminants Program was established in response to concerns regarding elevated contaminant concentrations in Inuit and biota of the Canadian Arctic. Fostering collaboration between communities and researchers has resulted in Arctic-wide monitoring programs that have established time-trends of persistent organic pollutants (POPs) and total mercury (THg) in arctic environmental media including wildlife since the early 1970's. How these trends have responded to climate change, an important issue in the Arctic (particularly in relatively low latitude locations) is not known to our knowledge, as many factors affect contaminant transport, deposition and retention in the arctic environment and ultimately, uptake in biota. The present study statistically modeled long-term trends of highly persistent and recalcitrant POPs (selected PCB congeners and the metabolite dichlorodiphenyltrichloroethane (DDE)) and THg in relation to climate-related covariates in polar bears (*Ursus maritimus*), thick-billed murre (*Uria lomvia*) and caribou (*Rangifer tarandus*), which occupy different habitats around Hudson Bay region. Here, the methods of variable selection, testing and model parameterization are outlined. Hudson Bay was selected as the study region as it is important for industry and development of the surrounding regions, is well trafficked, ranges into sub-Arctic regions and is a contamination "hot spot" relative to other Arctic sampling sites in Canada. Hudson Bay also has a relative abundance of environmental data available to the public. This allows for more detailed parameterization of the statistical models using variables and areas specific to the species of interest, which differ between bears (southern and western Hudson Bay), seabirds (Coats Island, northern Hudson Bay) and caribou (western Hudson Bay). Climate change-related data were available for a series of simple and complex variables e.g. air temperatures, wind speeds, precipitation, sea level pressure, ocean surface temperatures, sea-ice conditions and teleconnections such as the North Atlantic and Arctic oscillation indices. Assessments of the relationships between variables were undertaken and a series of models were developed for each species, limiting correlated factors while maximizing the independent variables used to parameterize them. Methodological and statistical challenges are highlighted, along with strengths and uncertainties of the resulting models.

WP003 Occurence and tissue distribution of organochlorinated compounds in Magellan penguin (Spheniscus magellanicus) from Southeastern Coast of Brazil

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Persistent organic pollutants (POPs), such as organochlorine pesticides (OCPs), are ubiquitous environmental contaminants of great concern due to their persistence, bioaccumulative and biomagnification potential and endocrine disrupting effects on both humans and wildlife. As a consequence of decades of intensive use, although banished and/or restricted in several countries, organochlorinated compounds (OCs), have been found in several environmental compartments, reaching even remote areas as a direct consequence of their persistence and ability to volatilize. Thus, the atmosphere and ocean currents are the main transport routes by which POPs enter pristine regions, although some studies have recently shown that it may also be transported to these regions via pelagic organisms and migratory birds. The presence of such compounds in birds from the Arctic and Antarctica regions is a clear evidence of this process, raising widespread concern about the concentrations of chlorinated compound residues and their detrimental effect on wildlife. Magellanic penguins (*Spheniscus magellanicus*) are known to migrate between Argentina and Brazil during winter, where they could be exposed to and accumulate high concentrations of anthropogenic pollutants. Being migratory and top predators in marine systems, penguins have been used previously as biomonitors concerning oceanic pollution in the marine ecosystem. In addition, seabirds present lower variability regarding contaminants in comparison to fish and marine mammals, allowing for the use of smaller sample sizes in environmental monitoring studies with a narrow confidence interval. The present study aimed to assess the levels and distribution of OCs in liver and breast muscle of penguins (N=25) found dead during regular beach monitoring at the Lake region in Rio de Janeiro, Southeastern Brazil. Only fresh carcasses were sampled. Approximately 2 grams of freeze-dried penguin tissue were extracted using an Accelerated Solvent Extraction method (ASE) based on the EPA Method 3545 and analyzed by GC-ECD. The main compounds detected were α- and β-endosulfan and their major metabolite, endosulfan sulfate, at high concentrations of up to 140 ng g^{-1}, clearly suggesting the presence of a polluting source of OCs in the studied area, which raises concern regarding the health risks these compounds pose to this threatened species.

WP004 Organic pollutants in Snow and Snow melting and its influence to High Arctic Lakes and Rivers

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The occurrence of legacy persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs; HCHs, DDTs and HCB) and other emerging pollutants such as perfluoroalkyl substances (PFAs) and organophosphate esters (OPEs) flame retardants and plasticizers was examined in snow, snow melting and water from two paired lakes and their main inflowing rivers located at the Cape Bounty Arctic Watershed Observatory (CBAWO), on Melville Island (74°55′ N, 109°35′ W) during 2015-2016. Samples were collected during pre-melting conditions, melting and open waters with the aim of study the main controls on the remobilization of organic pollutants into two lakes and their main tributaries at Cape Bounty. Legacy POPs, PFASs and OPEs were detected in all snow and water samples. Among the selected POPs, OPEs showed the highest concentrations with ΣOPE ranging from 15 to 40 ng/L, followed by PFASs and legacy PCBs and OCPs. Overall, the composition of the different OPEs, PFASs and legacy POPs were similar in the paired lakes and rivers, during the same sampling time. Dominant OPE congeners in lake waters were triphenylphosphate (TPP), tributylethoxy phosphate (TBEP) and tri(1-chloro-2-proply)phosphate. ΣPCB concentrations were in the range from 12 to 24 pg/L in lake waters and from 1.8 to to 3 ng/L in snow (meltwater) with the profile dominated by lower molecular weight PCBs homologues groups. The analyses of river and lake water samples showed the presence of PFASs at levels from 0.76 ng/L to 2.1 ng/L with a profile dominated by short chain, especially C4 PFCAs; PFOS was not detected. TPP was found to be the main contributor of OPEs in the lakes during open waters, which may be associated to snow melting. For legacy POPs, the melting and run off of snow during summer time was an important mechanism increasing the concentration of PCBs in high Arctic rivers up to 60 times in comparison to pre-melting and open water conditions.

Birds under Stress – Integrative Studies for Understanding Effects of Environmental Pollution in the Wild

WP005 Mercury Levels and Prevalence of West Nile Virus Antibodies in Songbirds Sampled from the Fountain Creek Region in Colorado

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Mercury is a neurotoxin that has been shown to affect brain activity and immune response in a variety of vertebrate species, including birds. One of the most common ways for mercury to be acquired is by diet. Dietary mercury exposure has been associated with suppressed immune responsiveness in captive song birds. However, no studies have tested whether exposure to mercury increases infection rates in wild songbirds. West Nile Virus (WNV) is a positive strand RNA virus (Flaviviridae) that is transmitted by mosquitoes (Culex species). The virus is normally maintained and amplified in avian reservoir hosts, but infected mosquitoes will also bite other vertebrates and can result in the transmission of the virus. WNV infections have been reported all over North America, including recent infections in Colorado. Mosquitoes are routinely sampled for the presence of WNV, but bird populations are more difficult to trap and analyze. In a collaborated effort, wild caught song birds have been trapped, banded, and blood samples were collected from birds in the Fountain Creek Region of Colorado in summers of 2014-17. Blood samples are now being screened for mercury levels using a DMA 3000 Mercury analyzer and for WNV antibodies using an indirect ELISA (enzyme-linked immunosorbent assay). We hypothesize that higher levels of mercury may correlate with WNV infection. Initial screening results show multiple birds being positive for WNV antibodies. Those same bird species have also shown higher levels of mercury than the other species. Over 800 bird blood samples have been collected over the last 4 years and will be analyzed for WNV antibodies and mercury. Geographic location, bird species, bird age, mercury levels, and WNV antibody titer data will be gathered and analyzed for any correlations between WNV exposure and blood mercury levels.

WP006 Climate variability and mercury levels in eggs of Arctic seabirds

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We investigated the influence of climate variability on total mercury concentrations (THg) in the eggs of northern fulmars (*Fulmarus glacialis*) and thick-billed murres (*Uria lomvia*) from Prince Leopold Island in the Canadian High Arctic. Weather and climate variables were compiled from a variety of in-field measured and satellite-derived sources, and included atmospheric data: air temperature, wind speed, and sea level pressure, oceanic data: sea surface temperature, primary productivity, and sea ice concentrations, atmosphere-ocean transfer data: snow and rain, as well as broad-scale teleconnection indices such as the Arctic Oscillation (AO) and North Atlantic Oscillation (NAO). Mercury monitoring data for the seabird eggs spanned 40 years (1975-2014); and climate influence time lags of 0 to 10 years were evaluated. The main effects of the weather and climate variables on THg concentrations were assessed using General Linear Modeling (GLM) and the most parsimonious models were selected for both seabird species using Akaike's Information Criteria (AIC). For both northern fulmars and thick-billed murres the most parsimonious models included NAO, precipitation, and temperature. Truncated datasets for the past 10 years, when THg concentrations in the eggs were relatively constant (2005-2014), were reasonably correlated with some climate variables such as precipitation. Overall, the results suggest a small, but significant effect of climate variables on THg concentrations in Arctic seabirds.

WP007 Mercury in Riparian Spiders and the Potential Risk to Appalachian Mountain Birds

A.C. Todd, G. Beaubien, C. Olson, R.R. Otter, Middle Tennessee State University / Biology

Mercury is a global contaminant capable of traveling long distances and depositing in remote areas generally thought of as pristine. Tennessee's Appalachian Mountains are a hotspot for mercury deposition (65billion mercury units per ½ hectare) and are host to, at some point in their life cycle, over 200 bird species. Birds can be exposed to aquatic mercury when they prey on benthic macroinvertebrates (i.e. mayflies, caddisflies, and midges) emerging from streams. Riparian spiders, specifically those that spin webs above the land-water interface and passively prey on emerging insects, have also been shown to serve as a vector for aquatic mercury, thereby extending the aquatic food chain and serving as an additional opportunity for mercury to biomagnify. The objective of this study is to determine the potential risk of mercury to birds that consume riparian spiders. To determine the viability of spiders as an exposure pathway of mercury to birds, in August of 2016, two groups of spiders (Families: Tetragnathidae and Araneidae) were collected from four streams that span the latitudinal gradient of Tennessee's Appalachian Mountains. Whole-body homogenates were analyzed for total mercury concentrations and compared to calculated total mercury spider-based avian wildlife values. The mercury concentrations of whole-body spider homogenates (Tetragnathids: 220 ± 28 PPB; Araneids: 85 ± 10 PPB) exceeded calculated wildlife values for at risk bird species such as the 1-day-old Carolina Chickadee (74 PPB) and 12-day-old Carolina Chickadee (49 PPB) at all sites. These results show that small passerine birds residing in areas thought of as pristine can still be exposed to physiologically significant levels of mercury if they consume riparian spiders.

WP008 Dose-Response Relationships for Metal Effects on Avian Reproduction: Cadmium, Selenomethionine, Vanadium, and Zinc

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We conducted dose-response analyses for effects of several metals on reproduction of birds, to support development of toxicity reference values (TRVs) suitable for application in a baseline ecological risk assessment. All studies were reviewed for appropriate study design, documentation, and data quality. We focused on test endpoints that are most directly relevant to reproductive success, including egg production, hatching success, and production of surviving offspring. For selenomethionine only, egg production was much less sensitive than offspring production, so selenomethionine studies evaluating only egg production were excluded from the evaluation. Egg injection studies were not considered, because egg injection is not an ecologically relevant exposure pathway and does not effectively mimic maternal transfer for metals. We also focused solely on laboratory studies using controlled experimental conditions due to the advantage of isolating the administered chemical as the cause of observed effects, and chronic exposures and multigenerational studies were preferred. Regression analyses were used to quantify dose-response relationships where sufficient data were available. Effects of cadmium, vanadium, and zinc on chicken egg production are well characterized, and dose-response relationships can also be defined for effects on egg production in Japanese quail (cadmium and vanadium only). Data on hatching success and chick survival are limited for these metals but suggest at most a modest increase in sensitivity compared to egg production. The lack of reproductive toxicity data for any bird species other than poultry is a major data gap that limits the ability to confidently estimate risks for wild birds exposed to cadmium, vanadium, or zinc. In contrast, reproductive effects of selenomethionine are well characterized for mallards, and data are also available for three other wild bird species. We present effects benchmarks and TRV ranges for these metals and discuss their application to assess risks to songbirds at metal-contaminated sites.

WP009 Effects of a hydrophilic organophosphate flame retardant, Triphenyl phosphate (TPHP), on corticosterone and stress-related behavior in Japanese quail

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Triphenyl phosphate (TPHP) is an organophosphate flame retardant that has been classified as a priority chemical under the Government of Canada's Chemicals Management Plan. It is widely used as a plasticizer and flame retardant. Preliminary studies have demonstrated that TPHP causes behavioural changes, physical deformities and alterations to stress axis (Hypothalamus-pituitary-interrenal (HPI) axis) gene expression in zebrafish (*Danio rerio)*. However, little is known about this compound's potentially toxic effects in other taxa, or the potential effects of its metabolites, e.g., Diphenyl phosphate (DPHP). We conducted a study with Japanese quail *(Coturnix japonica)* as a model, to evaluate possible effects of TPHP on birds. Developing quail were exposed to treatments *in ovo* and orally for the first week of life. Treatment groups consisted of safflower oil (control), environmentally relevant levels of TPHP (5 ng/g), higher levels of TPHP (50 ng/g and 100 ng/g), or a major metabolite of TPHP (DPHP, 100 ng/g). Doses of TPHP did not result in any dose-dependent effects on mortality and deformities. The birds' stress response was measured in 6-day-old chicks as a change in plasma corticosterone levels in response to handling. Baseline blood samples were collected within three minutes of entering the chick rearing room, and response samples were acquired 30 minutes later. These values were compared to behavioural measures of boldness, assessed as tendency to approach and make contact with a novel object, tendency to explore a novel environment, and tonic immobility (an extreme fear response). Increasing our understanding of endocrine and behavioural effects of TPHP in birds will support risk assessment to effectively minimize any potential deleterious impacts on wildlife.

WP010 Transcriptomics, contaminants, and other biomarkers in tree swallow (Tachycineta bicolor) nestlings on the Great Lakes and Maumee River, Ohio

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Legacy contaminants including polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), dioxins, and a variety of pesticides are significant issues in Areas of Concern (AOC) on the Great Lakes. Pharmaceuticals and personal care products have more recently been identified as contaminants of emerging concern (CECs) with limited knowledge of their effects through dietary exposure of terrestrial animals. Tree swallow (*Tachycineta bicolor*) nestlings were collected from 27 AOCs and nine nearby non-AOC sites from 2010 to 2015. In 2016, nestlings were collected from six locations on the Maumee River to evaluate CECs including pharmaceuticals and personal care products. Transcriptomic analysis was performed in nestlings collected from selected sites on the Great Lakes and the Maumee River. RNA-Seq library construction and sequencing was done with Illumina chemistries and the *de novo* assembly of RNA-seq data was completed with the Trinity platform, followed by RSEM for expression estimation and Deseq2 for differential expression analysis. Functional analysis was carried out by DAVID in combination with Pathway Commons to identify altered pathways and calculate Jaccard similarity between nestlings collected from different sites. Nestling gene expression variation among sites on the Great Lakes could be predicted from the amount of total PCBs, dietary PAHs, and perfluorinated compounds (PFCs); biomarkers related to DNA damage, cytochrome P450 1A activity, and oxidative stress were also highly correlated with transcriptomic responses which involved both aryl hydrocarbon receptor (AHR) – dependent and independent response pathways. Functional analysis further determined the correlation between chemical profiles, functional terms, and altered molecular pathways. The alterations in gene expression revealed potential adverse outcome pathways (AOPs) in nestlings, enabling the further assessment of chemical profiles, and providing insights into complex exposure scenarios.

WP011 Great Lakes colonial waterbirds as sentinels for continuing reproductive and health impairments at contaminated sites in Michigan during 2010-17

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This monitoring program assessed effects of contaminants, primarily PCBs and PCDDs, on immune function and reproduction in fish-eating birds in the Saginaw Bay and River Raisin Areas of Concern (AOCs) and Grand Traverse Bay during 2010-17 under the Great Lakes Restoration Initiative-Fish and Wildlife Service program. Saginaw Bay sites included two herring gull colonies (Confined Disposal Facility (CDF) and Little Charity Island), two Caspian tern colonies (CDF and Charity Reef/L.

Charity Island) and one black-crowned night heron colony (CDF). Herring gulls were studied in the River Raisin AOC at the Detroit Edison Monroe Power Plant on the western shore of Lake Erie and in Grand Traverse Bay on Bellow Island. Reference sites were in the lower St. Mary's River (gulls on Pipe Island Twins and terns on Two Tree Island) and on Chantry Island, Lake Huron (herons). Gull embryos were assessed during late incubation using a viability detector sensitive to heartbeat and movement. Embryonic nonviability in herring gulls in the Saginaw Bay and River Raisin AOCs (6.7% for the CDF, 6.8% for L. Charity, 8.6% for Monroe) and in Grand Traverse Bay (12.9%) was significantly higher than at the reference site (3.2%). Infertility was the primary cause of nonviability at the reference site. Elevated infertility and mortality contributed to nonviability in contaminated sites. Deformities associated with PCBs and PCDDs were found in several individuals at AOCs (3 gull chicks at Monroe, 2 tern chicks on L. Charity, 2 gull embryos on the CDF, and 1 gull embryo on L. Charity). In Saginaw Bay chick productivity was substantially below reference values in 3/6 years for terns on the CDF (with complete reproductive failure in 2015), 2/4 years for terns on Charity Reef and L. Charity Island, 1/7 years for gulls on the CDF, and 1/6 years for gulls on L. Charity Island. In the River Raisin AOC, productivity was very poor in 4/7 years, with complete reproductive failure during 2010. In gull chicks the mean phytohemagglutinin (PHA) skin response for T-cell mediated immunity was suppressed 52-55% at both AOCs and 46% in Grand Traverse Bay. This response was suppressed 46% in terns and 39% in herons in Saginaw Bay. Mean antibody responses were at least two-fold lower in herring gull chicks at both AOCs and in Grand Traverse Bay. Continuing immunological and reproductive impairments at these contaminated sites are consistent with the effects of persistent pollutants such as PCBs and PCDDs.

WP012 Spatial Trends of Organochlorine Compounds in Voyageur's National Park Using Nestling Bald Eagles as Indicators 2011-17

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Bald eagles (*Haliaeetus leucocephalus*) have been used as bioindicators of environmental contaminants in Voyageur's National Park (VNP) in MN, U.S.A since 1989. The eagle population in VNP is stable and the concentrations of many persistent organic pollutants (POPs) have decreased. However, previous studies indicated that some POPs are persisting or, in some cases, increasing within the park. The cause of this increase is unknown, suggesting that emerging sources of contamination may be influencing the ecosystem in VNP; therefore, we are continuing to monitor persistent organic pollutants in VNP in order to better understand their trends over time and across the spatial region. Twenty two polychlorinated biphenyls (PCB) congeners and twenty four organochlorine pesticides were measured in blood plasma. Compounds were separated from plasma using solid phase micro extraction and quantified using gas chromatography. ΣPCBs, ΣDDTs (dichlorodiphenyltrichloroethane and its metabolites), and Dieldrin were detected in >50% of plasma samples. The trends of these POPs have been delineated temporally and spatially. Preliminary results suggest that Rainy Lake has the highest contaminant load and variations in contaminant concentrations occur annually. It has yet to be establish what, if any, environmental factors initiate these fluctuations. The source of these fluctuations will be the subject of further analysis.

WP013 Avian and Mammalian Risk Assessments of Soil-Incorporated Pesticides

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The USEPA uses a modified version of the Hoerger and Kenaga nomogram for estimating potential exposure of birds and mammals to pesticides in its screening-level nontarget organisms risk assessments. This nomogram is generic and is based on residue data collected for foliar applications of a number of different pesticides applied to a variety of different crops and non-crops (e.g., turf). The methodology for estimating potential exposure of birds and mammals to pesticide residues consists of generic residue unit doses (RUDs) for different categories of crop or non-crops (e.g., grasses, broadleaf plants), or plant parts (e.g., seeds, pods, fruits). The generic RUD values for different categories of plants or plant parts are multiplied by the product application rate to estimate potential exposure, in mg/kg diet (ppm) of birds and mammals to that may ingest pesticide residues applied to the crop of interest. However, this methodology is inappropriate for estimating potential exposures of birds and mammals which may ingest plants or plant parts to soil-incorporated pesticides. Rather than the generic nomogram methodology, potential exposure of birds and mammals to soil-incorporated products depends on the properties of the product, such as whether the product is systemic, and translocation in the plant. A generic model for estimating potential exposure of non-target terrestrial organisms to soil-incorporated products is not available. Instead, estimates of potential exposures should be based on actual residue data for these products and crops they are used on. Rotational crop data can also provide useful insight into potential exposures of non-target organisms to soil-incorporated products. Examples will be provided for two soil-incorporated herbicides that contrast estimated residues on treated crops based on the standard nomogram methodology, and actual measured residues in these crops following soil-incorporated application. These data indicate that the nomogram methodology significantly overestimates exposure of non-target terrestrial organisms to soil-applied products.

WP014 Characterization of developmental effects of eight environmentally-relevant chemicals using an early-life stage Japanese quail toxicity test

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Currently, ecological risk assessment relies heavily on data from standardized tests that use invertebrate and piscine species. Although birds are a sensitive indicator of ecosystem health, avian toxicity data for many chemicals of environmental relevance are limited. The overall goal of our research project was to assess the effectiveness of avian early-life stage toxicity testing for rapidly screening chemicals of ecological and regulatory concern. Here, we utilized an avian egg injection protocol and used it to assess 8 chemicals (benzo[a]pyrene [BaP], lead (II) nitrate [Pb], seleno-L-methionine [SeMe], hexabromocyclododecane [HBCDD], ethinylestradiol [EE2], fluoxetine hydrochloride [FXH], trenbolone [TB] and chlorpyrifos [CPF]) that are commonly detected in Canadian ecosystems and have well-characterized modes of toxic action. The chemicals were dissolved in dimethyl sulfoxide and injected into the air cell of Japanese quail (JQ) embryos prior to incubation at three concentrations; the highest aimed to cause 20% lethality based on literature reviews. Liver tissue was collected from a sub-set (n=5/dose group) of embryos at mid-incubation (day 9) for subsequent 'omics and analytical chemistry analyses. The remaining embryos were examined on day 16 (1-2 days prior to hatch) for deformities, growth and health metrics. Mortality in the vehicle group was low across all experiments (3%), and was below the 20% target for EE2, CPF, and Pb (14, 9, and 6 % respectively). BaP was embryotoxic at the highest dose tested (0.5 µg/g) resulting in 50% mortality. EE2 and CPF exposure significantly reduced embryo growth (at concentrations of 33.3 and 40 µg/g egg, respectively) and CPF caused an increase in gallbladder size and increased incidence of deformities of the feet and spinal cord. Pb exposure resulted in a low occurrence of eye abnormalities at 1µg/g. Results for SeMe, HBCDD, FXH and TB are pending. Overall, the effects of chemical exposure in JQ were consistent with predicted toxicity profiles from other species. The use of avian embryos prior to hatch helps address the need to replace live animal tests with alternative approaches for chemical screening. The co-determination of apical and ‘omics end points will greatly contribute to ecological risk assessments and the development of adverse outcome pathways. This study is part of the EcoToxChip project (@ecotoxchip).

Assessing Contaminant Effects in Ecosystems with Multiple Stressors

WP015 A map of the Bayesian network relative risk model for incorporating adverse outcome pathways into a multiple stressor framework

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A challenge in environmental toxicology and risk assessment has been the extrapolation from the molecular interactions within an organism to landscape scale effects on population dynamics and community structure. The framework is derived from the Bayesian network relative risk model that has been used at a variety of scales, from watersheds to continental regions. Unfortunately it is difficult to clearly present in platform talks the details of both the construction of the network and the calcution. This poster presents the Bayesian network as described by Landis, Chu and others at SETAC North America 2017.The first step is the transformation of the required adverse oucome pathways into the basics of an Bayesian network. Our model is based on the AOP for organophosphates proposed by Ankely et al 2010 and Russom et al. (2014). The basic structure is similar but with outputs nessary to inform a population model to determine effects. The next step is that the AOP influence diagram is placed into an ecological risk assessment model based on the BN-RRM process as demonstrated by Landis and colleagues. The sources of the stressors are identified, the stressor exposures quantified and the exposure-response relationships are represented as the conditional probability tables in a Bayesian network. Other ecological factors such as water temperature, habitat quality and other features are represented by similar pathways. The results of these exposures are calculated for each of the endpoint, in this case Chinnok salmon. The example portrayed in this poster is for the effects of organophosphate pesticides in the Nooksack River of northwestern Washington State.

WP016 Assessing the Effects of Chemical Mixtures using a Bayesian Network-Relative Risk Model (BN-RRM) Integrating Adverse Outcome Pathways (AOPs)

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There are long-standing questions about toxicity of chemical mixtures to populations. Laboratory toxicity tests have confirmed synergistic and antagonistic effects to individuals, but not to populations. We have conducted a regional-scale ecological risk assessment by evaluating the effects chemical mixtures to populations with a Bayesian Network-Relative Risk Model (BN-RRM) incorporating an Adverse Outcome Pathway (AOP). We used this BN-RRM framework in a case study with organophosphate pesticide (OP) mixtures (diazinon, chlorpyrifos, and malathion) in four watersheds (Lower Skagit, Nooksack, Cedar and Lower Yakima) in the state of Washington (USA). Acetylcholinesterase inhibition (AChE) is the molecular initiating event and the Puget Sound Chinook salmon (*Oncorhynchus tshawytscha)* and Coho salmon (*Oncorhynchus kisutch)* Evolutionary Significant Units (ESU) were chosen as population endpoints. Laetz et al. (2009,2013) indicated that organophosphate pesticide mixtures act synergistically and impair AChE activity which leads to a change in swimming behavior and mortality, which then leads to changes in population productivity. Exposure-response equations have been generated for single chemicals, binary and ternary mixtures and integrated into the BN-RRM framework. Ecological stressors such as dissolved oxygen, temperature and habitat suitability were also included in our risk analysis. Overall risk results indicate that single chemical toxicological effects are just as important as ecological stressors. However, synergism between the binary and ternary mixtures changes risk. Synergism was most prevalent in binary mixtures containing malathion (malathion and diazinon, malathion and chlorpyrifos) and less prevalent in the binary mixture containing diazinon and chlorpyrifos. Ternary mixtures indicated about the same risk as binary mixtures containing malathion. This research demonstrates a probabilistic approach to estimate the effects of mixtures and predict impacts to populations.

WP017 Is the non-biting midge Chironomus riparius a suitable test organism to study the effect of multiple stress?

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Ecotoxicological standard tests that are used for the environmental risk assessment of chemicals exhibit limited environmental relevance. While organisms in the laboratory are exposed to a single stressor, organisms in the environment have to cope with a variety of biotic and abiotic factors. Some of them are known to increase the sensitivity of organisms to chemical exposure (e.g., thermal stress), while others even showed to compensate toxicity (e.g., higher food supply) - both can affect the risk assessment of chemicals. To examine whether the non-biting midge *Chironomus riparius* is a suitable test organism to investigate the influence of multiple stress, we conducted 28-days life-cycle tests (based on OECD guideline 219). Larvae were exposed to a wide range of different biotic and abiotic stressors (e.g., thermal stress, food deficiency, density, and chemical stress) with and without the addition of 0.4 mg/L (nominal concentration, representing the larval no observed effect concentration) of the anti-epileptic drug carbamazepine (CBZ). The aim of this pre-experiment was i) to identify stressors that are individually sub-lethal, ii) to investigate whether the sensitivity of the midges changes with increasing intensity of these stressors iii) and whether effects differ between observed endpoints and stressors. The results of this study will help develop a test method to investigate the impact of multiple stressors on the risk assessment of CBZ with *C. riparius*. Results of the currently running experiment will be presented on a poster at the conference. *Acknowledgement* - The authors thank the Federal Ministry of Education and Research (BMBF) for funding (NiddaMan, project support code: 02WRM1367A).

WP018 Sediment Toxicity Assessment in Two Wisconsin Areas of Concern and Selected Lake Michigan Tributaries

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Degraded benthos (bottom-dwelling or benthic invertebrates) is a widespread impairment at Great Lakes Areas of Concern (AOCs), typically caused by sediment contamination. Recent sediment toxicity data, however, were not available for the Sheboygan River AOC, where sediment remediation was completed in 2013, nor for the Milwaukee Estuary AOC, where remediation is in progress, or for non-AOC sites selected for comparison. In fall 2016, the USGS collected composite samples of surficial sediment from 19 tributary sites— 11 at the two AOCs and 8 at the non-AOC comparison sites. Sediment was evaluated for midge and amphipod toxicity, and for concentrations of PCBs, PAHs, selected metals (total metals and simultaneously-extracted metals [SEM]), acid-volatile sulfide (AVS), and organic carbon. Probable Effect Quotients (PEQs) for chemical groups were scored and summed to derive hazard rankings for each site. Highest overall hazard rankings were found in the Menomonee and Kinnickinnic Rivers of the Milwaukee Estuary AOC, whereas sites in the Sheboygan AOC had lower rankings, similar to the non-AOC sites. Amphipod toxicity at the sites was related primarily to PAHs and secondarily to metal concentrations in the sediment. For six sites with benthos community data from 2014, taxa richness correlated negatively with bioavailable metals (SEM normalized to AVS and organic carbon), and Index of Biotic Integrity values for benthos on artificial substrates correlated negatively with PCBs. Taxa richness of combined (dredge and artificial substrate) samples correlated negatively with sediment PCBs, PAHs, and metal bioavailability, as well as with hazard rankings. Overall, sediment toxicity, sediment chemistry, and benthic community data for these sites were in good agreement and provided a robust characterization of the AOC sites with completed and ongoing remediation efforts.

WP019 Spatio-Temporal Variations of Ecosystem Health Status During Last Three Decades in Tolo Harbor, Hong Kong, China

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Tolo Harbor is a nearly land-locked waterbody located to the northeast of Hong Kong with only one narrow exit open to the sea at Mirs Bay. With the rapid urbanization in its catchment during 1970s-1980s, Tolo Harbor had been suffered from various anthropogenic stresses. This caused the frequent occurrences of red tides and associated fish kills as well as coral deaths in Tolo Harbor in the late 1980s, which was referred as "Hong Kong's First Marine Disaster". An integrated action plan, Tolo Harbor Action Plan (THAP), was developed by the Hong Kong Government in 1988 to stop this disastrous trend. The present study was undertaken to evaluate the spatio-temporal variations of ecosystem health status (EHS) during last three decades in Tolo Harbor, using the dataset of long-term monthly monitoring program starting from 1986. The P-C-B triangle method was developed to perform this evaluation by integrating physical, chemical and biological indicators, and to identify the dominating factors. The results showed that, the EHS of Tolo Harbor would be divided into six periods with two deteriorations and two convalescences during 1986 to 2014. The first deterioration was caused by violent anthropogenic impact mainly from urban area. With the implementation of THAP, the EHS was significantly improved, and this convalescence was biological indicator-dominated. Later, the Harbor underwent a physical indicator-dominated deterioration, which was odd for normal expectation, since the anthropogenic emission was controlled by THAP. With the rapid decrease of physical indicator index in 2009, Tolo Harbor came into a stable healthy stage, and this convalescences was physical indicator-dominated. In addition, we also extracted the seasonal factors of long-term monthly time series. The results illustrated that the EHS had a bad tendency in most month. The ecosystem health tendency achieved the worst in April, and then this worse tendency maintained until June. From July, the ecosystem health tendency had greatly changed and maintained until March in the next year, only deteriorated temporally in September. Further analysis showed that this annually cyclic variation was highly related to biological indicator. In conclusion, the EHS variations in Tolo Harbor were well evaluated and the dominating factors were also identified by applying P-C-B triangle method, so we expect this method can be benefit for integrated environmental assessment and management.

WP020 Targeting Tilefish: Interesting Findings from a Circumnavigation of the Gulf of Mexico

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The Gulf of Mexico is a productive region for oil and natural gas extraction, fisheries, and tourism that receives continual riverine input and runoff from major agricultural and industrial regions in its bordering countries. This multitude of potential contaminants and ecosystem stressors, coupled with a dearth of baseline data in offshore teleost species, has complicated rapid health assessments after disasters like the Deepwater Horizon oil spill of 2010. Therefore, to understand the current state of demersal teleosts, a Gulf-wide survey of a long-lived, burrow-forming perciform with high site fidelity, the Golden tilefish (*Lopholatilus chamaeleonticeps*), was undertaken in 2015-2017, and included multi-year sampling of repeat sites in the northern Gulf. Fish tissue and blood were analyzed for traditional biomarkers and physiological evidence of toxicant exposure, including markers for: genotoxicity, oxidative stress, liver damage, and an activated immune response. These metrics were compared with body burdens of polycyclic aromatic hydrocarbons (PAHs), fish morphometrics, and environmental variables from sampling sites. Following preliminary data analysis, specific samples were chosen for transcriptome analysis to further expound gene expression changes that may correlate with predicted environmental PAH exposure regimes. In addition, field-caught Golden tilefish biomarker fluctuations were compared to those of a similar model species, Southern flounder, in a concurrent in vivo PAH dosing study at Mote Marine Laboratory. This paper will discuss work completed to date, noteworthy data anomalies from collection sites around the Gulf, and will highlight challenges remaining for this project.

Environmental -omics Measurements – Applications to Human and Ecological Exposure and Health

WP021 ToxMine: A novel toxicogenomics tool integrating chemical structures and gene expression profiles via network methods for improved toxicity assessment

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Here we introduce ToxMine as a unique online platform integrating diverse resources of toxicogenomics data to systematically characterize gene expression signatures and chemical structural features that are associated with different toxicity endpoints. By coupling several cutting-edge distance/similarity measures with network-based approaches, ToxMine allows researchers to quickly identify known or potential toxicity effects from multiple queries such as chemical names/structures, SMILES strings or list of significant genes. ToxMine considers best approaches to describe chemical structures and link them with designated expression signatures for a more efficient dose-response analysis. The underlying knowledge base is based on data curated from Tox21, ACToR, CTD, T3DB, LINCS L1000 and Open TG-GATEs with links to PubChem and other compound databases. ToxMine has been implemented as a high-performance cloud-based web application with an intuitive interface to allow users to easily browse, search, analyze and visualize their results to gain deep biological and mechanistic insights. ToxMine is designed for toxicologists, biologists, biochemists, and clinicians who are interested in getting useful information about possible adverse effects for a set of provided chemicals or gene signatures. ToxMine is freely available at http://www.toxmine.ca. This study is part of the EcoToxChip project (@ecotoxchip).

WP022 Assessing the biological impact of exposure to environmental surface waters by cell-based lipidomics

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Environmental surface waters often contain a variety of chemical contaminants from different sources including wastewater treatment plants, concentrated animal feeding operations, agricultural runoff and other human-related activities. Exposure to these contaminants may pose a threat to human health and the health of wildlife (e.g. fish). We have reported the use of cell-culture based exposures in monitoring the ecotoxicological effects of chemicals in the aquatic environment and our previous focus has been mainly on changes in polar and semi-polar metabolites (i.e. the metabolome) following exposure. In the current study, we extend this approach by focusing on changes in endogenous lipophilic compounds (i.e. the lipidome) of cells in response to contaminant exposure. Two human cell lines (HepG2 and LN229) and one fish cell line (ZFL) were exposed to surface water collected from 8 streams nationwide. These sites were selected from a larger study encompassing 38 sites that cover a variety of land-use types and contamination statuses. After a 48 hr exposure, the lipidome of control and exposed cells was profiled with Ultra-High Performance Liquid Chromatography coupled to a Q-Exactive Orbitrap Mass Spectrometer (Thermo Scientific). We analyzed the perturbed lipid metabolism pathways and identified potential biomarkers following the exposure to environmental contaminants. In addition, we used partial least-squares regression models to explore covariances among measured chemicals in the surface waters and the lipidomics dataset, providing insights into the ecological impacts and thus determining relevance of potential environmental contaminants.

WP023 A Study of the Environmental and Genetic Factors Affecting Antibiotic Resistance of Bacteria from Rio Grande River in El Paso, TX-Cd. Juarez, Mexico

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Background: The Rio Grande River provides a major source of potable and agricultural water for the population of the Texas/Mexico border region. Cattle farming and ranching are the most prevalent activities, which may contribute to the microbial burden and the release of antimicrobial agents and pharmaceuticals into our state's water resources. Antibiotics, presumably released into the environment by discharges originating from waste-water treatment plants, septic disposal systems, animal feeding operations and urban runoff, have a definite impact on the ecosystem and may contribute to an increase in antibiotic resistance. We hypothesize that waters of the Rio Grande River contain Multi Drug Resistant Organisms (MDRO), mobile genetic elements and antimicrobial organic residues. This could lead to a serious public health challenge for nearly three million people living along the El Paso / Juarez, Mexico border. Little is known about the degree of contamination and impacts of antibiotics in our region. The current study analyzed water samples collected along a 26 km segment of the Rio Grande River near a watershed and animal facility. The main objectives of this study are: 1) to isolate, identify and determine antimicrobial susceptibility patterns of bacteria, 2) determine the presence of antibiotic residues, and 3) determine the presence of genetic antimicrobial biomarkers. Methods: Isolation and identification of bacteria will be done by Microscan™, the presence of antimicrobial residues with HPLC/MS and the presence of genetic antimicrobial biomarkers by PCR. Results: Analysis of the first water samples collected in two different time periods tested positive for 6 isolates with resistant patterns. Positive genetic biomarkers for ESBL genes (TEM and CTX-M) and Integrons were identified in all the water samples and ESBL (TEM) genes were identified in the sediment samples. Also the MPN, a fecal indicator for water surfaces, was above the standards set by the Texas Commission on Environmental Quality. Currently, we are working on the identification of organic compound residues. Characterization of genes responsible for antibiotic resistance and the levels of 10 commonly detected antibiotics in the samples were studied and will be presented. The results of this study can lead to improved measures and policies in the management of infectious diseases and offer preventive measures in the antibiotic crisis we are facing worldwide.

WP024 Applying environmental omics to apportion the origin, chemical and toxicological profiles of organic aerosols

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Breathing air is contaminated by a spatiotemporally variant, complex, and heterogeneous mixture of gases and particles.Occupational and environment exposures to air pollutants induce adverse health effects (development/progression of sub-clinical and clinical disease, morbidity, and mortality) depending on composition (and size for particles), sources, and aging (physical and chemical transformations). Exposure characterization is instrumental in case-cohort and longitudinal epidemiological studies, and health risk assessments.The purpose of this study is to develop and apply a multivariate factorial analysis model to identify unique patterns and attribute them to OA sources/types. Traditional methods such as principal factor analysis (PCA) and/or discriminant analysis (DA) that are also used in environmental omics have limited applicability in natural sciences because of their infinite number of equivalent solutions and the presence of physically, and biologically, hard to interpret negative loadings. To overcome this, we implement a model that enforces non-negative restrictions and considers the influence of measurement uncertainty. We previously applied this modeling framework to resolve the sources of fine particulate matter, polynuclear aromatic hydrocarbons and volatile organic compounds in different environments. The functional and ^{13}C carbon isotopic composition of water soluble organic carbons (WSOC) in atmospheric aerosols were determined by NMR spectroscopy and isotope ratio mass spectrometry (IRMS) in an urban location in Southern Mississippi Valley. The origin of WSOC was resolved using the functional distribution of organic hydrogen, $\delta^{13}C$ ratios and Positive Matrix Factorization (PMF). Three factors were retained based on NMR spectral bins loadings and levoglucosan resonances. Differences between the two factors included the abundance of the aromatic functional group for factor 1, indicating fresh emissions and, for factor 3, the presence of resonances attributed to secondary ammonium nitrate and low $\delta\ ^{13}C$ ratio values that are indicative of secondary organic aerosol. Factors 1 and 3 added 0.89 and 1.08 µg C m^{-3} to WSOC respectively, with the highest contribution in the summer and fall. Factor 2 retained resonances consistent with saccharides and was attributed to pollen particles. Its contribution to WSOC varied from 0.22 µg C m^{-3} in winter to 1.04 µg C m^{-3} in spring.

WP025 Combining Equilibrium Sampling with Nontarget Analysis of Hydrophobic Complex Mixtures in a Complex Matrix

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Risk assessment of complex mixtures is a serious challenge. Typically, chemical analysis used in risk assessment is based on exhaustive extractions yielding total pollutant concentrations that do not reflect the actual contaminant exposure of organisms. In this study we combine equilibrium sampling with nontarget analysis to obtain extracts that reflect the available exposure in the matrix and obtain nontargeted "fingerprints" of the chemical composition in these extracts. Wastewater treatment plant (WWTP) sludge is the studied matrix. The equilibrium sampling was performed with jars coated on the inside with silicone. This technique provides some unique features for the subsequent instrumental analysis: the compounds are enriched by orders of magnitude in the silicone; typical interferences such as humic acids are excluded, and clean up steps are omitted. The aim of this study was: *i)* to compare the nontargeted chemical fingerprints from the equilibrium sampling with those obtained after exhaustive extraction, and *ii)* to compare the chemical fingerprints of exposure relevant hydrophobic compounds in sludge between and within WWTPs. Secondary and digested sludge were obtained from three Danish WWTPs. Biological activity was prevented by sodium azide and equilibrium sampling was conducted in jars coated on the inside with silicone in four thicknesses (2, 4, 8, and 16 µm). After equilibration the silicone was extracted. Without further treatment the extracts were analysed using full scan GC-MS EI. Equilibrium was confirmed by measuring target analytes as well as on nontarget basis by comparison of total ion chromatograms (TICs) from same sample equilibrated with different silicone thicknesses. Signal to noise ratio was reduced in the TICs from equilibrium sampling compared to exhaustive extraction, which is a clear advantage of this methodology. Equilibrium sampling revealed clear differences in the mixture composition between WWTPs in the secondary sludge, whereas this difference was not apparent for the digested sludge. Some compounds like acids are removed or reduced, while e.g. sterols seemed to be up concentrated during the treatment process. This pilot study shows the potential of combining equilibrium sampling on complex environmental matrices with a nontarget analytical approach.

WP026 Trends of known and new contaminants of emerging concern measured in a wastewater-influenced stream using passive samplers

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Integrating passive samplers, such as the polar organic compound integrative sampler (POCIS), provide the means to collect an integrated profile of many polar organic contaminants present in surface water whose presence may be continuous or episodic, reflecting changes in hydrologic or source input. POCIS permits contaminant trend assessment by (1) identification and quantitative concentration using methods for targeted contaminants, such as analgesics, antidepressants, stimulants, and their degradates, as well as (2) identification and semiquantitative estimation of nontargeted contaminants using a comprehensive screening strategy. We present results from structured target and nontarget analysis of POCIS extracts deployed over a year-long period under differing flow conditions. The extracts were analysed using a Quadrupole Time-of-Flight Mass Spectrometer (QToF MS) operated in the positive electrospray ionization mode and coupled to an ultra performance liquid chromatograph, using a C-18 reversed phase column for separation of analytes. The QToF MS was operated in a scan mode combining spectra with alternating low and high collision cell energies. The resulting data were then aligned and analysed instrument software and an extended pesticide and toxicology library to identify known and suspected contaminants. Results from the QToF analysis were compared to targeted analysis using high-performance liquid chromatography/tandem quadrupole mass spectrometry (LC/MS/MS). Methyl-1H-benzotriazole, carbamazepine, citalopram, desvenlafaxine, fexofenadine, and tramadol were among the compounds in POCIS extracts detected by LC/MS/MS and confirmed by QToF MS. Lamotrigrine and DEET, not determined by LC/MS/MS but anticipated to likely be present, were identified by QtoF MS from elemental compositions of the parent pseudomolecular ions and the associated fragments from the linked high-energy collisional dissociation spectra. The hydroxyl degradation products of carbamazepine and lamotrigrine were similarly identified in these extracts, as were ketamine, which was not initially expected to be present. Trends in the relative abundances of suspected and newly identified contaminants were consistent with those observed for targeted compounds by LC/MS/MS. The complementarity of targeted and nontargeted analysis is also well demonstrated by the trends in relative abundance under differing flow conditions and in different sections of the stream reach.

WP027 Diversity of chemicals in house dust explored using suspect screening and non-targeted analysis

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The exposome encompasses the totality of exposures to all stressors for an individual over time. Measuring and understanding the exposome is important to more fully understand the origins of disease, and to ensure that chemicals in commerce are safe. Given that humans spend the majority of their time indoors, and that children have increased exposures due to child-specific activities and behaviors, it is critical to understand exposure to house dust and all of the chemicals contained therein. A pilot study was undertaken to characterize the dust exposome, and whether chemicals found in house dust can indicate exposure to non-chemical stressors (e.g., poor nutrition). Suspect screening and non-targeted analysis were used to examine dust samples collected from house dust stratified by floor (upstairs, downstairs), individual rooms (13 total), size fraction (< 150 µm and 150 µm-1 mm), and collected at three distinct time points. Dust samples and QA samples (blanks, replicates, NIST SRM 2585) were extracted in methanol and analyzed using liquid chromatography (LC) with high resolution accurate mass (HRAM) quadrupole-time of flight (QTOF) mass spectrometry (MS). Thousands of unique aligned features (constituting peaks at a retention time, with mass spectra and abundance) were identified, matched to the USEPA's Distributed Structure-Searchable Toxicity (DSSTox) database, and further investigated using the USEPA's CompTox Chemistry Dashboard. Spearman correlation coefficients showed strong agreement between features across the two size fractions ($r > 0.95$), and good agreement between features in upstairs vs. downstairs samples ($r > 0.85$). Based on tentative chemical assignments, the chemical stressors in highest abundance are classified as flame retardants, plasticizers, pesticides and personal care products. Further investigations will include the analysis of single room and additional time point dust samples to focus on: 1) whether granularity of sampling variables impacts chemicals detected and/or identified; 2) whether or not tentatively-identified chemicals can be used as indicators of non-chemical stressors; and 3) the likely sources of compounds detected (chemical and non-chemical stressors).

WP028 Genomic Analysis and Functional Characterization Reveal Similar Soil Microbial Community Shifts in Biodiesel vs. Petroleum Diesel Contaminated Soil

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Genomic studies have been increasingly used in environmental measurements to fulfill our understanding of chemical risks to microorganisms. Although biodiesel has been considered as a safe substitute for petroleum diesel, the degree to which biodiesel is more soil microbe-friendly than petroleum diesel is inconclusive. This study set out with the aim of revealing the temporal changes in composition and function of soil microbial communities to biodiesel and petroleum diesel and detecting whether soil microbial communities can revert to initial compositional and functional states after a significant degradation of biodiesel and petroleum diesel. Sandy loam soils were manually contaminated with one of three types of biodiesel or a low sulfur petroleum diesel and incubated in the laboratory for 180 days. Soil microbial composition changes were addressed by 16s rRNA gene sequencing of the V3-V4 region while function diversity was examined by commercial Biolog EcoPlates™. The degradation of contaminants was determined by gas chromatograph-flame ionization. Although biodiesel degraded faster than petroleum diesel, results suggest that biodiesel and petroleum diesel impose similar selective pressure on soil microbial communities and there was no clear suggestion that biodiesel contaminated soil microbial communities can revert to predisturbance states within the 180-day timeframe. After half-year natural attenuation, the compositional diversity of soil microbial communities was reduced in both petroleum diesel and biodiesel treatments, and the functional evenness of both petroleum diesel and biodiesel was significantly lower than uncontaminated treatment. In conclusion, the genomic study and the EcoPlate™ approach reveal that biodiesel is not more microbe-friendly than petroleum diesel.

Alternative Approaches to Animal Testing for Ecotoxicity Assessments

WP029 A novel physiologically based pharmacokinetic (PBPK) model for perfluorooctanoic acid (PFOA) in male rats

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Perfluorooctanoic acid (PFOA) is an important global contaminant with unique toxicokinetics. Previous research has indicated that the tissue distribution and elimination of PFOA in mammals are influenced by interaction with different proteins including serum albumin, fatty acid binding proteins (FABPs) and organic anion transporters (OATs). By taking protein interactions into account, we have proposed a novel permeability-limited physiologically based pharmacokinetic (PBPK) model to estimate the toxicokinetics of PFOA in the male rat. Specifically, our model considers PFOA association with serum albumin in vascular and extracellular spaces, association with intracellular proteins (i.e., liver-type FABP and α2μ-globulin) in liver and kidney, and cellular uptake and efflux via passive diffusion and active transport facilitated by OAT proteins. The model is evaluated using measured tissue distribution and toxicokinetic datasets collected from three different studies, where different dose levels and administration routes were used. Comparing the model prediction with experimental data shows that our model performed well in predicting PFOA toxicokinetics within a factor of 5, with most experimental data falling within the range of predicted values. Unlike most current PBPK models that rely on experimental data fitting for parameterization, all 72 parameters used in our model were extrapolated from the literature (e.g., in vitro studies for protein interactions and cellular uptake, physiological data for the rat). This makes our model a promising tool in predicting PFOA toxicokinetics in humans and informing risk assessment given that experimental data for humans are scarce.

WP030 Characterizing the mechanism of androgen-induced activity in the luciferase transactivation VM7Luc4E2 cell bioassay

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The chemically-activated luciferase expression (CALUX) VM7Luc4E2 cell bioassay has an estrogen receptor-driven response element and is used for screening estrogenic chemicals. Indeed, the VM7Luc4E2 has been certified by the Organisation for Economic Co-operation and Development in 34 countries for detection of estrogenic or anti-estrogenic substances and is included in the Tier 1 U.S. Environmental Protection Agency's Endocrine Disruptor Screening Program and multi-agency Tox21 Program for high throughput screening of chemicals for estrogenic/anti-estrogenic activity. However, it has been previously documented that testosterone can induce luciferase activity in this cell line. If the cell line is an accepted in vitro method for screening of estrogens, we thought it prudent to explore in further detail this seeming agonist activity from androgens in the VM7Luc4E2 cell line. Here, we characterize the activity of several androgens in the VM7Luc4E2 cell line and potential mechanisms by which androgens exert activity in the VM7Luc4E2 cell line.

WP031 Developmental effects of maternally transferred selenium to early life stage fathead minnow (Pimephales promelas)

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Selenium (Se) is a naturally occurring essential trace element that can persist and bioaccumulate in aquatic systems. Selenium also displays a narrow range between dietary essentiality and toxicity and has become a contaminant of concern in North America. Anthropogenic activities such as mining and agriculture result in leaching and mobilization of inorganic Se into surface waters where it is assimilated and biotransformed by algae and microorganisms to organic Se (e.g. selenomethionine [SeM]). Primary and secondary consumers facilitate trophic transfer of SeM throughout the food web and ultimately SeM can bioaccumulate to significant concentrations in higher trophic level taxa. Oviparous vertebrates are particularly sensitive to SeM toxicity, and maternal transfer of SeM is a key route of Se exposure in early life stage fish, which can lead to teratogenic effects in many species and can potentially elicit deleterious population level effects. Unfortunately, this process is challenging to study in long-lived species of concern that inhabit North American ecosystems. The objective of this study was to develop and validate an embryo injection approach to model maternal transfer of SeM. This model could then be applied to any species of interest and could provide insight regarding species sensitivity differences for Se toxicity in early life stage fish. Initially, the maternal transfer of dietary SeM and its effect on the F1 generation were characterized in a short-lived species native to North American freshwater systems, the fathead minnow *(Pimephales promelas*). Specifically, 120 breeding pairs were fed a SeM-spiked diet (0, 5, 15, 45 g/g d.w) and bred for 45 days. Average Se embryo concentrations from this study were then used as the basis for subsequent embryo injection studies in fathead minnow. Endpoints used to compare toxicity of SeM between the reproductive and microinjection assays included hatchability success, percent mortality, and total deformities. Linear regression was utilized to compare Se embryo concentrations to early life stage toxicity to determine whether similar *in ovo* Se concentrations produce similar toxic effects in both assays.

WP032 ecodatahub: Big data techniques to create actionable ecotox data

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Recent advances in computer technology allow data collection and integration at speeds and in volumes greater than ever before. These advances, coupled with increasing data and meta-data generation, have initiated the "big data" revolution, while the field of data analytics has emerged to aid in the evaluation, interpretation, and visualization of massive data sets. Disciplines such as marketing, social media, and geospatial climatology have led the charge, applying concepts of big data to address complex questions. Many scientific fields have yet to utilize the benefits of big data applications but instead continue to rely on entity-level data approaches, which typically consist of an individual (or company/organization) storing databases on computers using platforms like Microsoft Access or Excel. While these data may be highly vetted and reliable, they often lack the volume, diversity, and density available from an integrated, multi-sourced dataset. Evaluating the pros and cons of entity-level and big data approaches makes clear that a hybrid approach, which incorporates the best of both strategies, is ideal and feasible. The ecodatahub proof-of-concept project aims to develop, build, and implement this novel hybrid approach for ecotoxicological data. Essentially this project aspires to combine the reliability of entity-level approaches by using experts in the field to source data, and determine data quality, while concurrently using big data techniques and data analytics to manage, integrate and visualize data. The user-friendly ecodatahub interface will be free and publicly available so that users can easily interact with datasets and data streams. Current regulatory and legislative frameworks drive the need for robust ecotoxicity data generation and analysis, while stressing reduction in animal testing. ecodatahub will address one of the biggest limitations related to development of new, alternative methods: the lack of access and integration to existing datasets that include the raw data needed to evaluate new methods. The project is supported by a multi-sector, multi-stakeholder group via financial and in-kind contributions, and is advised by a team of leading experts in toxicology, exposure science, chemistry, computer science, and risk assessment. This poster will highlight the overall hub concept, providing an overview of the strategy, proof-of-concept project, and future directions.

WP033 Ecological Threshold for Toxicological Concern (eco-TTC): Exploring the importance of non-standard species

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The Threshold for Toxicological Concern (TTC) is well-established for assessing human safety of indirect food-contact substances and has been applied to a variety of endpoints. Recently, we have proposed an extension to the human safety TTC concept for environmental applications, termed the ecological TTC (eco-TTC). The strengths and limitations of an eco-TTC approach are still being investigated. Algal tests are an important component of chemical environmental risk assessments and are the most sensitive taxon approximately 50% of the time. The complete eco-TTC database contains approximately 120,000 toxicological records (tests) employing some 2500 different species. Further, the eco-TTC database contains over 14,000 curated records from 300 unique algal species dominated by standard test species such as the green algal genera *Pseudokirchneriella, Scenedesmus*, and *Desmodesmus*, the marine *Skeletonema* and *Phaeodactylum* and the blue-green *Microcystis* and *Anabaena*. Here, we explore how hazard values for final PNEC derivation may change with the inclusion of standard and non-standard algal test species supported by analyses of eco-TTC and USEPA Web-ICE (Web-based Inter-species Correlation Estimation) applications. An eco-TTC derived hazard value will also be compared against an algal SSD for data rich chemicals such as cadmium chloride, triclosan, and a cationic surfactant. This work was performed with input from the HESI Animal Alternatives in ERA Technical Committee. The views, conclusions and recommendations expressed in this article are those of the author and do not necessarily represent views or policies of the USEPA.

WP035 Identifying chemical features associated with molecular initiating events for impaired vitellogenesis

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High-throughput screening approaches have provided toxicity data for large chemical inventories. Structural analyses have also been useful for identifying chemical features leading to adverse responses. Within the adverse outcome pathway framework, such information can be useful to discern the mode of action of a substance when several putative molecular initiating events (MIEs) exist. For example, there are multiple endocrine MIEs (i.e., androgen receptor agonism, estrogen receptor antagonism, aromatase inhibition) associated with reduced synthesis of vitellogenin, the egg-yolk precursor protein. These changes can lead to reduced fecundity and reproductive dysfunction in fish, thus affecting population viability. However, several alternative modes of action (i.e., mitochondrial dysfunction, oxidative stress) can also lead to impaired vitellogenesis. Since these alternative pathways may confound results in fish reproduction assays, it is important to characterize predominant MIEs leading to toxicity. The objective of this study was to identify chemical features leading to unique MIEs using machine learning. Hit calls below cytotoxicity were extracted for 10 Tox21 assays describing 7 MIEs leading to impaired vitellogenesis. Substances tested in all assays (5374) were parsed using the Chemical Subgraphs and Reactions Markup Language for 729 ToxPrint chemotypes. These ToxPrints were then used to develop a classification model predicting hit calls in each of the Tox21 assays using a Random Forest algorithm. With common parameters, the out-of-bag estimated error rate was highest for the model predicting hits in the TOX21_ARE_BLA_agonist_ratio assay (14.57%) and lowest for the TOX21_AR_BLA_Agonist_ratio assay (1.47%). After each model was developed, the algorithm evaluated the importance of each ToxPrint chemotype to identify critical structural features leading to unique MIEs. It was found the “ring:hetero_[5]_N_imidazole” ToxPrint was most predictive of aromatase inhibition, while also being highly predictive of oxidative stress. In addition, the “ring:fused_steroid_generic_[5_6_6_6]” ToxPrint was most important for androgen and estrogen receptor agonism, and “ring:aromatic_benzene” was most important for aryl hydrocarbon receptor agonism and mitochondrial dysfunction. These data suggest chemical structures are useful for discerning putative MIEs leading to impaired reproduction in female fish.

WP036 New(ish) tools in the toolbox:Using in vitro bioassays for cumulative assessment of steroid hormone receptor active compounds in environmental samples

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Numerous monitoring studies have been conducted globally demonstrating the utility of in vitro assays for providing a quantitative assessment of cumulative receptor-based activity from samples with broad chemical contamination. The consensus is that in vitro bioassays can provide value-added information regarding the types and levels of contaminants that may be present but may or may not be detected by traditional analytical chemical determinations in an environmental sample. Both proprietary and publically available cell lines/assays covering multiple receptor types are available for use by researchers, regulators, and those conducting primary monitoring of water treatment processes. Our research group has specifically utilized the T47D-KBluc and MDA-kb2 cell lines for detection of estrogen and androgen receptor activity, respectively. More recently we have interrogated environmental samples using CV-1 cells virally transduced with either glucocorticoid or androgen receptors and accompanying response elements and reporters. Virally transduced cells offer some advantages to stably transfected cell lines such as higher levels of reporter gene expression above background and greater control of receptor/reporter expression across multiple cell passages. We have successfully utilized these assays in collaborative studies with USGS on stream water, drinking water, and wastewater effluent in national water quality screening efforts. Across studies, estrogen receptor activation was detected with the highest frequency. Concentrations of estrone typically explained the majority of ER activity (≥90%), with more minor/sporadic contributions of estriol, 17β-estradiol, and 17α-ethinyl estradiol. Further, we observed lower frequency of detection of AR or GR agonism and in most cases the paired chemical analyses did not provide detections of known AR or GR active compounds. Detection of receptor antagonism is considerably more challenging than agonism and must be optimized for the concentration of competitive ligand and paired with additional assays, such as sample cytotoxicity, to determine if true receptor antagonism is present. Moving forward, a greater understanding of comparative inter-assay responses, and in vitro-to-in vivo extrapolation of effects are needed to establish rigorous trigger values and propel in vitro bioassays into standardized usage in water quality monitoring regimes. Abstract does not reflect the views or policy of USEPA.

WP037 Optimization and Accessibility of an Ecotoxicological Threshold of Concern (ecoTTC) Database

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The Ecological Threshold for Toxicological Concern, or ecoTTC, has been proposed as a natural next step to the well-known human safety TTC concept. The ecoTTC is particularly suited for use as an early screening tool in the risk assessment process, in situations where chemical hazard data is poor, or when an appropriate QSAR is unavailable. EcoTTCs are developed using statistical distributions of Predicted No-Observed Effect Concentrations (PNECs) to reflect the breadth and depth of the ecotoxicological dataset beneath, and therefore, the diversity and quality of the underlying dataset is crucial to the future utility of the ecoTTC. A database consisting of approximately 110,000 unique ecotoxicological records, 6200 unique CAS numbers and 1900 species from three trophic groups has been created based on recent assessments of published data and international chemical management programs. Stepwise data selection strategies, query systems and curation techniques were applied to ensure a transparent, methodical process towards a final dataset, which also includes reference-sourced toxicity data associated with physical chemistry data and taxonomic information for the tested chemical. In order to make these data accessible and useful to stakeholders, the dataset was transitioned from Microsoft Excel and Access into a modern MySQL format. This allows for a database format that is relational and scalable, facilitating easy access, sharing, and integration with other datasets and tools. This dataset is accessed via a web-based query system that is integrated with PNEC calculator and probability distribution tools. The novel interface allows users to explore the data, upload additional datasets, derive threshold values based on specific criteria, and explore the potential use and application of the ecoTTC concept. This poster will present the architecture, web-interface, and associated tools and a live demonstration of the web interface, and associated web tools will be available.

WP038 Predictive approaches for assessing environmental hazards of industrial chemicals

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Predictive methods such as in-silico [quantitative structure-activity relationship (QSAR) and read across] tools and in-vitro methods are efficient and cost-effective approaches for assessing the environmental hazards of industrial chemicals in terms of their persistence, bioaccumulation or toxicity (P,B, or T) potential. These predictive methods can be used at various phases of a chemical product development ranging from screening and prioritizing during early research and development phase to fulfilling regulatory requirements for product registration. Three separate case studies with different focus will be presented to exemplify the application of these tools for assessing chemical safety. The first case study illustrates the application of computational (in silico) tools to assess environmental hazards of research samples (research sample safety datasheet). The second case study illustrates the application of in-silico approach (e.g., Read Across) combined with medium throughput screening assays to assess the biodegradability and potential aquatic toxicity of new products during early stages of development. The third case study illustrates the application of in-silico approaches to support the registration of difficult-to-test substances (e.g., highly insoluble compounds). These case studies demonstrate the utility of in silico predictive approaches with various degrees of uncertainties.

WP039 Scoring oxidative stress in fish cell lines via a novel in vitro assay

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Establishing in vitro methods is considered a high priority, since most risk assessment relies on in *vivo* studies. The Swedish Research council - amongst other European institutions - is increasingly funding programs dedicated to 3R principles. Therefore, we started our project "toxicity pathways – a novel strategy to reduce and replace in vivo studies in fish". It is the prescribed goal to establish a battery of stably transfected *D.rerio* cell lines in order to test for specific toxicity endpoints. The latter may then be used for omics-based high throughput applications, screening, and prioritizing of positives. First, transfection efficiency of commercially available transfection reagents (Mirus X2, LT1, 2020; Promega FHD, F6, Viafectamine, TransFast; Roche XHP; Invitrogen Lipofectamine 2000; Qiagen SuperFect, Effectene) were tested in Pac2, ZF4, and ZFL *D.rerio* cell lines. Most promising candidates (FHD for Pac2 and ZF4, XHP for ZFL) were selected for cell transfection in transient reporter gene assays. A Nrf2-sensitive pGL4-based firefly-luciferase expressing plasmid was co-transfected with a normalizing renilla-luciferase plasmid. Second, all three reporter lines were exposed for 24h to exponentially increasing concentrations (0.1, 1, 10, and 100 µM) of known oxidative stress inducing chemicals (tertButylquinone, peroxide, and sulforaphane). In parallel, cell-viability was scored using standardized MTS-assay. Dose-response relationships could be shown in all transiently transfected cell lines for tertButylquinone and sulforaphane. Concentrations of causing less than a 20% decrease in cell-viability were considered as non-cytotoxic and should be used in ongoing assays for receptor activation or inhibition. ZF4 and ZFL were prioritized for further use. Last, a panel of environmentally applied pesticides (diazinon, deltamethrin, atrazine, metazachlor, tert-Buthylazine, diuron) was used for 24 h exposure (6.25, 12.5, 25, 50, 100 µM) of Nrf2 transiently transfected cells, in order to test for the ecotoxicological applicability of the bioassay. Dose response relationships were shown for certain tested pesticides (metazachlor, deltamethrin, diazinon), proofing the robustness of the assay.

WP040 The Utility of Chinese Rare Minnow and Chinese Medaka in Ecotoxicology: Prospects for Fish Embryo Testing and Interspecies Correlation Estimation

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Future environmental hazard assessments for chemical registrations in China routinely involve fish testing. Testing of fish must be done in the country using locally available and relevant species is a priority. The Chinese Rare Minnow (CRM, *Gobiocypris rarus*) and other native species (Chinese Medaka, *Oryzias sinensis*) are examples of leading local species being considered. Both species are not available for testing and investigation outside of China at this time. Furthermore, animal testing concerns for fish comes into play and the Fish Embryo Test (FET, OECD TG 236) and flexibility to accommodate new species is foreseen and written into the Test Guideline. Our research first aimed to develop an understanding of the ecology and life history, physiology, and other basic biological information which is critical in the extrapolation between these two species and other global, common test species (e.g., fathead minnow, zebrafish, Japanese medaka). Second, we investigated fish acute toxicity by CRM in this phase on common testing chemicals: 3,4-Dichloroaniline and NaCl with 96 h LC_{50}s of 5.07 (4.31~5.96) mg/L and 9,485 (8006~11220) mg/L respectively, which will be followed by further ecotoxicity studies on additional chemicals and species. These results are in similar ranges to those previously published using the zebrafish FET. The studies in our laboratory can be incorporated into future research towards the development of Chinese Rare Minnow and Chinese Medaka FET assays. Last, Chinese native species data will be utilized for the development of statistical extrapolation methods, such as interspecies correlation estimation (ICE) models by using available but limited toxicity data of surrogate species to predict untested species, a valuable exercise expected to great expand the domains of ecotoxicological information for China not only for environmental hazard assessments but help developing environmental quality criteria, as lacking toxicity data of native species remains as a key challenge for such effort.

Current and Future Challenges in Sediment Toxicity Testing for Environmental Risk Assessment

WP041 Effects of Acid Volatile Sulfides (AVS) from Na2S-Amended Sediment on Hyalella Azteca

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Hydrogen sulfide is a relatively common sediment toxicant generated by microbial processes and important for interpreting sediment toxicity testing results. Ratios of acid volatile sulfides (AVS) and simultaneously extracted metals (SEM) have been used extensively for predicting bioavailability of divalent metals (i.e., Cd, Cu, Pb, Ni, Zn) in sediments of aquatic environments. The aim of this research was to measure relationships of AVS (as sodium sulfide [Na_2S]-amended sediment) and toxicity to a sensitive benthic amphipod *Hyalella azteca* to evaluate the exposure-response relationships among a series of sulfide exposures. The specific objectives were to (1) measure SEM/AVS ratios in a series of sodium sulfide ($Na_2S{\cdot}9H_2O$)-amended sediments producing a range of sulfide concentrations and (2) measure responses of *H. azteca* (as mortality) in 96-h static sediment toxicity tests to exposures of Na_2S-amended sediments. Amended sediments had a predictable increase in AVS concentrations and a concomitant decrease in ΣSEM/AVS ratios. Increasing concentrations of AVS resulted in a range of ΣSEM/AVS ratios that varied over more than an order of magnitude from 0.185 to 0.006. *H. azteca* survival decreased with increasing concentrations of "excess" AVS, with 96-h no observable effect concentration (NOEC) and LC50 of 0.041 and 0.019 ΣSEM/AVS, respectively. Clearly, the SEM/AVS model provides a useful tool for evaluating potential bioavailability of divalent metals and predicting ecological risk; however, this study demonstrates the need to consider sulfide (as AVS) as a potential source of toxicity in situations with low [

WP042 Establishing the Functional Equivalency of the Negative and Solvent Control for EPA 10-Day Spiked Sediment Testing in Support of FIFRA Registration

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OCSPP guidelines 850.1735 and 850.1740 (spiked whole-sediment 10-day toxicity tests) recommend the use of both a negative control group and a solvent control group when a volatile solvent is used to spike the sediment with the test substance. However, when the solvent is evaporated completely, as recommended in the OCSPP 850.1000 guideline, the Agency will allow the use of only one control (solvent) to be used, if the testing laboratory can demonstrate the functional equivalency of the negative and solvent controls. The EPA suggests using the following four factors when determining equivalence between the negative control and solvent control: (A) Concentration of solvent in the test sediment after evaporation (e.g., analytically determined); (B) Levels of the solvent that are known to affect organism health; (C) The potential for impurities in the solvent and their potential impact on organism health and; (D) Historical organism performance of solvent vs negative controls. Our study will evaluate historical relative performance of the negative control and solvent control, as well as seek to determine the concentrations of the solvent (acetone) that may interfere with the performance of the organisms tested (*C. dilutus, H. azteca, L. plumulosus*). The study will be conducted as a solvent spiked sediment exposure. The results of this study will establish whether standard testing procedures may result in potential solvent interference, and if not, establish functional equivalency of the two controls, and validate the use of only one control (solvent) during 10-Day sediment testing.

WP043 Are Residues of Solvents used for Spiked Sediment Toxicity Tests a Biological Concern?

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The physicochemical properties that make compounds potential sediment contaminants are often the same properties that limit their water solubility. As such, conducting spiked-sediment studies frequently requires the use of solvents for stock preparation to ensure that test material is fully dissolved; thereby, allowing for accurate dosing of test sediments at targeted concentrations. However, unlike during water-only toxicity tests in which organisms are concurrently exposed to both solvent and test material in the test solution matrix, test organisms in sediment toxicity tests are never directly exposed to the solvents. Rather, solvents are only used as transient carriers. This can be better understood by briefly describing the general sediment-spiking process. Due to low water solubility, test material is fully dissolved in a volatile solvent, often acetone, to create a concentrated stock(s). A measured aliquot of the required stock is then evenly distributed to a sand substrate. The active ingredient-coated sand is then allowed to dry for a period of time, mixed with the remaining test sediment components, and allowed to equilibrate to achieve nominal concentration. The hypothesis examined here is that the solvent used during the spiking process is evaporated to levels that are not biologically relevant, thus leading to an exposure scenario in which solvent has no effect. However, the U.S. Environmental Protection Agency guidelines for sediment-spiked testing (OCSPP 850.1735 and 850.1740) require concurrent negative and solvent controls during tests. To test our hypothesis, an experiment mimicking the acetone stock delivery and evaporation procedure used for spiking sediment was performed, and the loss of acetone from sand was quantitatively measured at different intervals using headspace gas chromatography (GC) with flame ionization detection (FID). This study was performed according to good laboratory practices (GLP) and was designed to have applicability for both freshwater and marine sediment tests using either natural or synthetic sediment. The findings of this study and implications for preparing spiked-sediments for toxicity tests will be discussed in detail.

WP044 Comparison of the use of natural vs. formulated sediment in sediment toxicity testing

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Formulated sediments are mixtures of materials that mimic the physical components of natural sediments. Formulated sediments provide a basis by which any testing program can assess the acceptability of their procedures and facilities and provides a consistent measure of evaluating performance-based criteria necessary for test acceptability. The use of formulated sediment eliminates interferences caused by the presence of indigenous organisms, eliminates variation in sediment physico-chemical characteristics and provides a consistent method for evaluating the fate of chemicals in sediment. An acceptable formulated sediment should (1) support the survival, growth, or reproduction of a variety of benthic invertebrates, (2) provide consistent acceptable biological endpoints for a variety of species, and (3) be composed of materials that have consistent characteristics (consistent from batch to batch, contaminant concentrations below concentrations of concern and ingredients available to all individuals and facilities). Natural sediments should (1) come from a clean source, (2) be fully characterized, and (3) free of organisms that might compete with or consume the test organisms. An examination was performed on data generated byEAGLaboratories-Easton on the ability to meet control validity criteria for a variety of species used in sediment toxicity tests and specifically a comparison was made on which sediment type (natural vs. formulated) was used during the testing. Additionally, a comparison was made on the characterization of formulated and natural sediments used in the conduct of these studies. Similar results between sediments were observed for acute tests while some of the chronic tests performed dramatically better with natural sediments.

WP045 Toxic evaluations with fish embryos in sediments collected from Osaka Bay, Japan

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Chemicals derived from human activities have been carried in coastal area by rainfall, river water, and others and settling directly from atomosphere. Then, they are settled on coastal sediments finally. Despite being habitats of numerous aquatic organisms on and/or in sediments, we have only poor knowleadge for actural toxcities of real environmtnal sediments polluted by chemicals. In fact, it's difficult to know the effects only from sediments, excluding contribution of chamicals dissolved in upper layer water, because benthic organisms can not exist without upper water. We consider that sediments are composed with soil and pore water, and have established to keep Japanese medaka (*Oryzias latipes*) embryo on the sediments without the raring water. Osaka Bay closes to big citeies as Osaka and Kobe cities, and has been polluted by several kinds of chemicals. In the present study, the toxicities of coastal sediments collected from 26 sites in Osaka Bay 2014 to 2016 were evaluated with medaka embryos. Sediments were sifted and then dired. Then, they were washed by embryo raring mediums (ERMs=pore water). Ten embryos were embedded in half of them in sediments and stayed at 24°C for 7 days. Control group with silica sand was also prepared similar with coastal sediment groups. After exposures on sediments, all embryos were transerred into 48 well-plates set ERMs with each one embryo/well, and reared there for other 13 days. We examined the effects such as mortality, hatching rate, hatching day (dpf), and inducing rate of malformation on larvae after hatching. Although sediments seriously affected to medaka embryos were limited, sediment collected near sewage plant was killed 13% embryos. This was suspected to be resulted from the chemicals in discharge water from the plant. Only 60% embyors exposed to sediment collected at river mouth of Yodo river, which flows in Osaka city, were hatched for 20 dpf, although their mortarity was only 3.3%. They could be affected to chemicals carried by river water. Additionally, sediments collected from several sites caused hatching delaies from 1 to 6 dpf compared with control group.

WP046 Relative sensitivity of Daphnia magna and Hyalella azteca to polycyclic aromatic hydrocarbon in water-phase and whole sediment toxicity tests

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Poorly water soluble chemicals are tend to be accumulated in the sediment and suspended solid due to its hydrophobicity, so it is important to evaluate sediment toxicity. There are three methods for testing sediment toxicity for environmental risk assessments: the equilibrium partitioning method, the pore water method, and the whole sediment toxicity test using benthic organisms. In the equilibrium partitioning method, sensitivity of the pelagic and benthic species is considered as similar and the sediment toxicity is estimated from toxicity value for the pelagic species, which may possibly underestimate/overestimate toxicity to benthic organisms. Additionally, it excludes the exposure pathway through direct contact and ingestion of sediment by benthic organisms. Since few studies have been conducted to investigate the sensitivity comparison of the pelagic and benthic species and various exposure pathways, in this study, we conducted acute toxicity test using a pelagic species *Daphnia magna* and a benthic species *Hyalella azteca* in both water-phase and spiked whole sediment exposure system. We selected polycyclic aromatic hydrocarbons (PAHs) as a model group of hydrophobic chemicals. The toxicity of a four-ring PAH pyrene in the whole sediment exposure was higher than that in the water-phase exposure to both *D. magna* and *H. azteca*. The similar trend was demonstrated with 1-aminopyrene and a five-ring PAH benzo(a)pyrene. The experiments to compare the toxicity in spiked sediment (e.g., OECD TG218) and in spiked in water with sediment (e.g., OECD TG219) is also being conducted. We are also planning to conduct similar experiments for additional chemicals to clarify the overall trend of the sediment toxicity of hydrophobic chemicals.

WP047 Evaluating the toxicity of Northeastern USA stream sediments with the amphipod Hyalella azteca, the midge Chironomus dilutus

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As part of the US Geological Survey's National Water-Quality Assessment Program (NAWQA), a total of 52 sediment samples representative of urban and agricultural land use were collected in the summer of 2016 to be evaluated under the Regional Stream Quality Assessment project. Relationships between biological conditions and concentrations of contaminants, metals, pesticides, PAHs, nutrients, and sediment in streams from the northeastern Region (CT, MA, NH, NJ, NY, PA, RI) of the United States were evaluated by conducting whole-sediment toxicity tests. The sediments were tested in accordance with ASTM and USEPA methods for conducting sediment toxicity tests with the amphipod *Hyalella azteca* (28-d exposure), and the midge *Chironomus dilutus* (10-d exposure). Toxicity endpoints included survival, weight, and biomass. Mean control survival at the end of the exposures met test acceptability criteria. Statistical comparison to the control identified 52% of the sediments (27 of 52 sites) as toxic to at least one of the test species, as determined by a significant reduction of at least one endpoint relative to the control sediment. Amphipod exposures identified 44% (23 of 52 sites) of the sediment samples as toxic. Midge exposures identified 16% (8 of 51 sites) of the sediments as toxic. Results from the 2016 study of the Northeast will be compared to other NAWQA regional sediment-toxicity surveys conducted in the Midwest (2013), the Southeast (2014) and the Pacific Northwest (2015). Ongoing efforts including development of a reference envelope approach to compare organism response to reference sediments, and comparisons between sediment toxicity and sediment chemistry, will be presented.

WP048 Use of Long-Term Sediment Toxicity Tests to Characterize Impacts of Metal-Contaminated Sediments on Benthic Communities

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Sediments from a 220-kilometer reach of the upper Columbia River (UCR) in Washington USA, including Lake Roosevelt National Recreation Area, are contaminated with metals originating from historic smelting operations. Previous studies have documented sediment toxicity in the UCR using short-term survival and growth tests with the midge, *Chironomus dilutus* and the amphipod, *Hyalella azteca*. We conducted long-term (42-50 d) tests with these species in 54 sediment samples to evaluate whether long-term endpoints would provide additional information on the extent and severity of sediment toxicity in the UCR. In addition, this testing was intended to support the development of sensitive and reliable site-specific thresholds for predicting metals toxicity. Toxic effects on amphipods occurred primarily in sediments from the riverine (upstream) reach of the UCR, whereas toxic effects on midges also occurred in the downstream (reservoir) reach. Toxicity in the upstream reach was associated with sediments containing greater concentrations of copper and other metals associated with slag from smelter operations. Reservoir sediments contained lesser concentrations of copper and zinc, but lead and cadmium concentrations remained elevated in both sediment and pore water in the reservoir. Based on a reference-envelope analysis, endpoints from 42-day amphipod tests classified about twice as many sites as toxic, compared to 28-day survival and growth tests, but endpoints from midge life-cycle tests identified about the same number of sites as toxic, compared to 10-day tests. Concentration-response models for the most sensitive short-term amphipod endpoint (28-d biomass) produced EC20 values for sediment copper that were 3-8 times greater than EC20s for the long-term endpoints, including survival (day 42) and reproduction. Our results demonstrate the value of long-term amphipod testing, but support previous findings that that long-term midge test protocols may not provide enough additional information to justify their additional cost. The different responses of these two test organisms demonstrated the advantages of testing with a suite of test organisms to better reflect patterns of exposure and toxic effects of contaminated sediments on benthic invertebrate communities.

WP049 Ecotoxicological risk assessment of metal contaminated boreal lake sediments with Lumbriculus biotests

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There is a pressure for new mining activities in many boreal regions since the production of new technological devices requires huge amounts of metals. Even though the mining technologies have developed and efforts are made to reduce the environmental impacts of mining operations, the recovery rate of these remote areas is slow. Boreal lakes in Finland are characterized by soft water with low buffering capacity. This makes them sensitive to excess ion loading. Ecotoxicological risks of two separate mining sites were assessed with hypolimnion and sediment field sampling and a chronic toxicity test using a laboratory reared freshwater Oligochaeta *Lumbriculus variegatus*. This species can be used as a good representative of the freshwater benthic fauna. In total, 12 mining-affected lentic locations from two separate mining sites and two reference lake sites in Finland were sampled. Water metal concentrations were compared with environmental quality standards (EQSs) and sediment metal concentrations with a national guideline for dredged sediments. The national EQSs for water were mostly not exceeded during our sampling time, except for soluble nickel or cadmium concentrations in two high mining impact sites. Comparisons of sediment metal concentrations with the national dredging guideline suggested that the main contaminants were Cd, Ni and Zn in the lakes of the first mining site, and Cd, Cu and Zn in the sites impacted by the other mining site. Oligochaetan *L. variegatus* were exposed to field collected sediment with hypolimnion or artificial fresh water (AFW) above the sediment. Results showed that the growth and reproduction were inhibited when *L. variegatus* were exposed to the natural field collected sediment together with hypolimnion in the medium or high mining impact locations. Response was not as evident with AFW. During the toxicity tests a drastic drop in pH was observed. Our study brought out the challenges of assessing contaminated sediments together with hypolimnion as they are. The objective was to overcome limitations related to application of standardized biotests to field-collected sediments in order to gain a precise case-specific ecotoxicological risks assessment of the two different mining sites. We discuss the pros and cons of methodologies besides results indicating deterioration of environmental quality of boreal lakes downstream the mining sites.

WP050 Sediment composition influences toxicity of naphthalene sulfonates to Tubifex tubifex

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Naphthalene sulfonates (NSAs) are incorporated into a variety of commercial products (e.g., dispersant in pesticide formulations, lubricants) and produced in relatively large volumes in North America. Based on their physical and chemical properties, NSAs could be potentially persistent, bioaccumulative and/or toxic, consequently, the Government of Canada is assessing this class of compounds under the Chemicals Management Plan for potential risks to the environment. The current study assessed the toxicity of three NSAs (calcium dinonylnaphthalene sulfonate (CaDNS), barium dinonylnaphthalene sulfonate (BaDNS), and 4,5-dinonylnaphthalene-1,7-disulfonate (DNDS) to the freshwater oligochaete worm *Tubifex tubifex*. Mature worms were exposed to two types of sediment spiked with each NSA for 28 days in aerated (static) vessels with a 3.5:1 water-to-sediment ratio. Survival of mature worms, cocoon production, and juvenile worm production were assessed following exposure. Preliminary data analysis based on nominal concentrations showed that LC50s varied significantly among the three NSAs examined. For example, in sediment with organic carbon content of ~2%, CaDNS and BaDNS (LC50s < 2,000 µg/g dry weight (dw)) were significantly more toxic to mature worms than DNDS (LC50 > 10,000 µg/g dw). Reproduction was a significantly more sensitive endpoint than mature adult mortality with EC50s for CaDNS and BaDNS of < 200 µg/g dw and for DNDS of > 1,000 µg/g dw. The composition of the sediment also had a significant effect on the toxicity of all three NSAs to *Tubifex tubifex*. A negative relationship was observed between organic carbon content of sediment and toxicity. The results of this study will support environmental risk assessment activities to determine if NSAs could impact freshwater ecosystems.

Systems Biology for Ecotoxicology – From Gene to Ecosystem

WP051 -Omic Analysis of the Impact of Lithium Nickel Manganese Cobalt Oxide (NMC) Nanomaterial on Shewanella oneidensis MR-1

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Nanoparticles have become crucial components in many current technologies and are increasingly being integrated into new designs. Unfortunately, nanoparticles have become widely incorporated before policy for their disposal was created and their environmental impact understood. Because of the high potential for environmental exposure, it is important to investigate the impact of nanoparticles on bacteria due to the various critical roles they play to maintain a healthy ecosystem. The interaction of interest in this work is the effect of lithium nickel manganese cobalt oxide (NMC) nanomaterial on *Shewanella oneidensis*. NMC is a novel, battery cathode material found in high quantities in emerging car technologies. *S. oneidensis* has the ability to reduce a variety of metal ions extracellularly, allowing it to interact with the heavy metals of NMC. Previous work has shown that NMC nanoparticles are toxic to *S.* oneidensis, but little is known about the mechanism of these negative effects. The interface of nanomaterials and bacteria can be probed using a variety of bioassays and mass spectrometry-based -omics techniques. In combination, both of these approaches have been used to understand both the perturbation of bacteria biochemical pathways and population-level phenotypic responses. Bioassays have been used to assess the global health of the exposed cultures and give insight into the ability of

S. oneidensis to develop resistance to NMC exposure. Multiple -omics approaches have been used to elucidate this resistance and expand on the mechanism of toxicity of NMC. Using a variety of techniques allows for the discovery of the toxic properties of nanoparticles and gain deeper insight into the mechanism of toxicity and recovery of an organism upon exposure to nanoparticles.

WP052 A method for CRISPR/Cas9 mutation of genes in fathead minnow

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CRISPR/Cas9 genome editing allows for the disruption or modification of genes in limitless model organisms. Here, we discuss the method as developed for use with the fathead minnow (*Pimephales promelas*), in part to assist in validation of adverse outcome pathways (AOPs). Successful generation of CRISPR/Cas9 mutants in fathead minnow requires identification of a target gene, design of guide strands specific to the target, microinjection of Cas9/guide strand complexes into one cell embryos, growth and observation of phenotype, and finally genetic analysis of mutants. The tyrosinase gene was the initial target chosen for development and assessment of the method since its disruption results in abnormal pigmentation, a phenotype obvious within 3 to 4 days after injection of fathead minnow embryos. Three tyrosinase-targeting guide strands were generated using the fathead minnow genome browser (setac.org/fhmgenome) in tandem with crispor.org. The strands targeted sequence coding for the calcium binding domain and the N-terminal region of the tyrosinase domain; both regions are found in the first half of the gene. In order to generate one cell embryos, in vitro fertilization (IVF) was performed using a protocol developed for zebrafish (zfin.org). This allowed for microinjection of hundreds of developmentally synchronized embryos with Cas9 proteins complexed to each of the three guide strands. Fish were grown and observed for abnormal pigmentation and eventually anesthetized, photographed and flash frozen. DNA extraction of the larvae was followed by amplification of the potentially mutated region by PCR, and analysis of amplicons by sequencing or endonuclease assay to assess mismatches. This method greatly advances our ability to directly investigate gene function in fathead minnow, allowing for a more nuanced approach to AOP validation and development. It could also potentially produce novel molecular level insights into the basic biology of fathead minnow and other fishes. The contents of this abstract neither constitute nor necessarily reflect official USEPA policy.

WP053 Assessing the health of wild yellow perch (Perca flavescens) populations from the St. Lawrence River, Canada, using a RNA-sequencing approach

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To understand the in situ cumulative effects of human and natural perturbations on freshwater organisms, yellow perch (*Perca flavescens*), an ecologically-relevant non-model species, were collected along the St. Lawrence River (SLR), Québec, Canada. The objectives of this study were to identify impacted biological pathways in three different yellow perch populations of the SLR exposed to a gradient of regional environmental stressors, using high-throughput RNA-sequencing to screen the transcriptome and link these transcriptomic responses to cellular activities, tissue integrity and general conditions. Yellow perch were collected in 2014 in the upstream fluvial Lake Saint-François (LSF) with the lowest degree of environmental stressors; Lake Saint-Louis (LSL) considered having a moderate degree of perturbations and Lake St. Pierre (LSP), a sector where the yellow perch population has been declining. Results indicated that fish from the downstream LSP showed lower body condition, compared to LSF and LSL, suggesting that this population of yellow perch were in lower global health. Furthermore, large differences in liver gene expression profiles were found among fish populations. Namely, 280 genes were over-expressed in LSP fish while 200 genes were under-expressed, compared to LSF and LSL perch where transcriptomic responses of fish were similar. RT-PCR analyses were conducted to validate RNA-seq results. In fish collected from LSP, transcript involved in growth, reproduction, retinol metabolism, oxidative stress, pentose phosphate pathway, immune system were more abundant, compared to those of LSF and LSL, suggesting that multiple metabolic and physiological pathways may be impacted by site-specific environmental stressors. Biomarker analyses (i.e., enzyme activities, protein content) were conducted to complement genomic results. Overall, impacted biological pathways found in the present study for LSP yellow perch may help understand the precarious state of this fish population that has led the provincial government to impose a moratorium on the sport and commercial fishing of this species in this sector of the river. Further investigations are underway to link changes of yellow perch transcriptome profiles to specific environmental stressors.

WP054 Characterizing early changes in molecular toxicity pathways to predict adverse outcomes of ethinyl estradiol and chlorpyrifos in amphibians

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Amphibians are of vital importance to aquatic ecosystems and are generally regarded as a sensitive bioindicator of ecosystem health. Exposure to contaminants can have adverse outcomes on amphibian health, such as altered rate of metamorphosis, reproductive effects, immune suppression, and behavioural effects. Alterations in molecular processes often precede these apical effects giving early indications of subsequent physiological changes and their modes of action. Toxicogenomics therefore shows great promise as an early screening tool to prioritize chemicals with potential risk for adverse effects, without the need for long-term, animal-intensive exposures. The main goal of this study is to identify and validate key molecular toxicity pathways that are predictive of contaminant-induced apical responses in the model amphibian, *Xenopus laevis*. Specifically, this study focused on chlorpyrifos (CPY) and ethinyl estradiol (EE2), two anthropogenic contaminants of concern with different modes of action and characterised apical effects in amphibians. Post-hatch individuals were exposed for 96 h to CPY (0.5, 2, 8 ug/L) and EE2 (0.04, 0.2, 1 ug/L) and sampled for whole transcriptome (RNASeq) and mass-spectroscopy-based shotgun proteomics to characterize their molecular toxicity pathways. A subset of tadpoles was then transferred to a flow-through diluter system for exposure to metamorphosis (~ 40 d) and assessed for developmental stage, morphometrics, organ histopathology and genetic sex. We anticipate that this work will identify critical toxicity pathways and associated specific key genes to be used in an early life-stage gene expression assay to predict apical outcomes of ecological and regulatory relevance in amphibians, providing an alternative approach in chemical screening. This study is part of the EcoToxChip project (@ecotoxchip).

WP055 Large scale application of chemical-gene interaction networks to connect field analytical chemistry with biological consequences

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The development of chemical-gene interaction networks provides a new tool in the assessment of environmental contaminants. They are particularly useful where complex mixtures of anthropogenic chemicals are the major source of stress to aquatic organisms. The approach matches lists of detected chemicals with genes and biological pathways, known to interact with the specified chemicals. Chemical analyses are among the most common, critical, and first tools deployed for environmental assessments. Chemical-gene interaction networks provide links between complex chemical mixtures in the environment and the biological implications of exposure. The information generated can be useful for hypothesis-driven assessment approaches and identifying appropriate types of bioassays. They can also provide a means to identify gaps in the available bioassays. To examine the utility of this approach we used the chemical dataset from the recent USGS “Chemical Mixtures” study (38 sites, ~800 chemicals). Mining genomic databases identified 300,000 unique chemical-gene interactions. From this baseline dataset, chemical-gene interactions were pared down to represents chemicals specific to each site. Site specific lists ranged from 4 – 250 detected chemicals. For each site, genes and biological pathways (KEGG pathways) related to chemicals were identified. All sites were evaluated through cluster analysis and principal component analysis (PCA) to identify patterns and commonalities among genes and pathways interactions. Further, pathways were grouped into broad biological response groups, BBRGs: cancer, disease, endocrine, genetic, immune, metabolic, neurologic, regulatory, and signaling. This helps to identify which types of mode-of-action specific testing may be most useful. Chemical concentrations were also integrated into the assessment, identifying the concentration of chemicals likely to interact with a given gene, pathway, or BBRG. A number critical pathways and genes, known to be “adverse” in dysregulation, were assessed across the sites. Additionally, efforts were made to connect this assessment to responses from bioassays done on these same sites. This provides a key connection between the chemical-gene predicted biological responses and those measured in the bioassays. While this type of evaluation is in its early stages, it represents a step towards integrating chemical and biological responses to understand complex mixtures of environmental contaminants.

WP056 Linking CYP1 Gene Expression and PAH Metabolic Profiles to Elucidate Mechanisms of Synergistic Toxicity

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous, non-point source aquatic contaminants derived from various combustive, fossil fuel, and urban runoff sources. PAHs’ toxic mechanisms, especially in complex mixtures, are poorly understood. Cytochrome P450 enzymes (CYP), particularly those in the CYP1 family, are extensively studied enzymes involved in xenobiotic and drug metabolism. The enzyme cytochrome P45001A (CYP1A) has long been recognized for its role in the oxidation and detoxification of certain PAHs; however, it was discovered recently that PAH toxicity is not directly related to CYP1A induction, and some PAHs, particularly tricyclic PAHs, act via distinct pathways and cause developmental cardiotoxic effects in fish. To elucidate these mechanisms and develop diagnostic biomarkers to assess the health of fish populations, a simplified model of an environmentally-relevant PAH mixture was examined by combining phenanthrene, a tricyclic PAH recently shown to disrupt cardiac function in developing fish, with chrysene, a four-ringed PAH metabolized through the aryl hydrocarbon receptor (AHR)-CYP1A mediated pathway, in developing zebrafish embryos (< 48 hpf). Preliminary work in this model has revealed a significant decrease in heart rate when in the presence of phenanthrene with increasing doses of chrysene by 48 hpf ($p < 0.05$), suggesting synergistic toxicity of this dual PAH mixture. The current study explores how CYP1 gene expression and PAH metabolite composition change leading up to this 48 hpf timepoint upon exposure to phenanthrene, chrysene, and mixtures of both. Expression of the CYP1 family of enzymes (CYP1A, CYP1B, CYP1C1, CYP1C2, and CYP1D1) was quantified using reverse transcription polymerase chain reaction (RT-qPCR). To quantify phenanthrene and chrysene metabolites in this time series, embryos and water from the exposures were hydrolyzed to convert phase II PAH metabolites to phase I metabolites and subject to liquid chromatography tandem mass spectrometry (LC-MS/MS). By linking gene expression with metabolic profiling, this time series will reveal the role of specific CYP1 enzymes in the metabolism of phenanthrene and chrysene, further elucidating information about the synergistic toxicities of PAH mixtures. Full results forthcoming.

WP057 Non-AHR Dependent Mechanisms of Toxicity: Genetic and cellular markers of mitochondrial oxidative stress in zebrafish exposed to furan-analogs

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Widespread production of alternative energy sources may include new untested chemical formulations with unknown environmental effects. New formulations of greener compounds are less persistent in sediment and may potentially be less toxic; however, additional research is needed to assess the toxicity of these compounds to aquatic organisms. The aim of this study is to examine toxicity of furan-based biofuels and combustion products using zebrafish, an established aquatic toxicological study organism. Because of similar binding of the AHR receptor to aromatic compounds in both mammals and fish, we first explored if the zebrafish aryl hydrocarbon receptor with highest TCDD affinity, zfAHR2, was induced in zebrafish. We hypothesized that CYP450s will cause toxicity from mono-oxidation reactions from phase I detoxification enzymes. Oxygenated metabolites play a role in oxidative and mitochondrial stress. Results from our assay for AHR activation indicated that toxicity to zebrafish from 9 furan chemicals demonstrated non-AHR dependence or non-AHR agonist mechanisms. The chemicals screened were a candidate biofuel, 2,5 dimethyl furan, and its combustion byproducts: 2,-3, hydrofuran, 2-ethylfuran, 2-methylfuran, 2-pentylfuran, 2,3-dimethylfuran, 2,5-dimethylfuran, furfural, indene, furan, as well as a potent AHR agonist, betanapthoflavone (BNF). To assess the link between chemical structure and conserved non-AHR pathways, we measured transcription from Nrf2 targets, and oxidative stress genes. Both Nrf2 and Gst are activated by exposure to electrophiles and protect against oxidative stress. Markers for mitochondrial cellular stress (ATP, oxygen demand, and mitochondrial proton gradient) were significantly altered by compound compared to controls. Our results permit a novel ranking of biofuel combustion products and AHR indicate that non-AHR pathways are differentially induced by chemical structure.

WP058 RNA-Seq Analysis to Identify Potential Biomarkers and Molecular Signaling Pathways in Japanese Medaka exposed to 17- α-Ethinylestradiol

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Estrogenic contaminants are continuously released into water bodies, causing alterations in the reproductive morphology and physiology of aquatic organisms. Exposure of fish to 17-α-ethinylestradiol (EE2), a well-studied synthetic estrogen agonist commonly found in sewage effluents, is known to elicit gonadal abnormalities, ranging from intersex to complete sex reversal. These gonadal changes have been well-studied in Japanese medaka, a laboratory fish model with genetic sex determination. Our objective was to identify molecular biomarkers for estrogenic (EE2) exposure and to understand the molecular signaling pathways involved in the resulting development of gonadal intersex and sex reversal. Medaka larvae were exposed to 0, 30 and 300 ng/L EE2 for 15 days, from 7 dpf to 22 dpf (days post fertilization). Exposure to these concentrations during the sex differentiation period is known to elicit the development of gonadal intersex and complete sex reversal, respectively. At the end of the exposure period, larvae from different groups were collected and processed for next-generation sequencing (RNA-Seq) using Illumina HiSeq2500. Tophat and Cufflinks were used to identify differentially expressed genes, and Ingenuity Pathway Analysis (IPA) was used to identify affected gene networks and functional pathways. Genes involved in sex differentiation and gonadal development, such as *gsdf*, *cyp19b*, *des*, *hsd3b7*, were significantly deregulated. Several of the prominently affected signaling pathways involved genes associated with steroidogenesis and steroid metabolism, such as *cyp2b3*, *cyp3b40*, *cyp1a*, *hsd17b4*. Genes associated with coagulation, FXR/RXR and LXR/RXR activation networks were also significantly altered. Our findings will improve our understanding on the molecular changes associated with the development of gonadal abnormalities after EE2 exposure.

WP059 The use of adverse outcome pathway-based toxicity predictions - A case study evaluating the effects of imazalil on fathead minnow reproduction

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Adverse outcome pathways (AOPs) provide a framework supporting greater use of mechanistic data measured at lower levels of biological organization as a basis for regulatory decision-making. An AOP description linking inhibition of aromatase (cytochrome P450 [cyp] 19) to reproductive dysfunction was reviewed for scientific and technical quality and endorsed by the OECD (https://aopwiki.org/wiki/index.php/Aop:25). High throughput screening in USEPA's Toxcast program identified the azole fungicide, imazalil, as an endocrine active chemical capable of inhibiting mammalian cyp19 and likely 17α-hydroxylase/17,20lyase (cyp17) in vitro. Based on these results, imazalil was selected as a case study chemical to test an AOP-based hazard prediction. Twenty-four hour exposures with fathead minnow (*Pimephales promelas*), focused on effects on production and circulating concentrations of 17β-estradiol (E2), key events in the AOP, were conducted to verify in vivo activity. A computational model of the fish hypothalamic-pituitary-gonadal-liver axis and a statistically-based model of oocyte growth dynamics were used to predict impacts of different concentrations of imazalil on multiple key events along the AOP, assuming continuous exposure for 21 d. Results of the model simulations were used to select test concentrations and design a fathead minnow reproduction study in which fish were exposed to 20, 60, or 200 µg imazalil/L for durations of 2.5, 10, or 21d. Within 60 h of exposure, female fathead minnows showed significant reductions in ex vivo production of E2, circulating E2 concentrations, and significant increases in the ovarian expression of mRNA transcripts coding for cyp19a1a, cyp11a (cholesterol side-chain cleavage), and cyp17. A concentration-dependent decrease in cumulative fecundity was also detected for fathead minnow pairs exposed continuously for 21 d. Overall, results of the study provide strong support for the qualitative relationships represented in the AOP and provide further evidence of concentration-response and temporal concordance among key events. The quantitative models were generally consistent with in vivo potency, supporting the use of this established AOP linking aromatase inhibition to reproductive impairment in fish as a means for predictive risk assessment. The contents of this presentation neither constitute nor necessarily reflect USEPA policy.

WP060 Toxicogenomic characterization of the effects of EE2 and FLX in fathead minnows: Identifying signature toxicity pathways to predict adverse outcomes

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Pharmaceuticals and personal care products (PPCP) are becoming an increasing concern to water managers due to their toxicity to non-target organisms, pseudo-persistence and continuous discharge into surface water systems. Among these chemicals are the endocrine disrupting compound 17α-ethinylestradiol (EE2) and the selective serotonin reuptake inhibitor fluoxetine (FLX). Little is known about the (sub-)chronic effects of these compounds to aquatic organisms that are chronically exposed to low concentrations. In particular, information is limited on the molecular pathways that drive cascades of events that ultimately result in phenotypic adverse outcomes. Recent advances in next generation 'omics technologies provide a platform for the unbiased characterization of toxicity pathways in target organisms as they allow entire biological systems to be probed without a priori knowledge of the mechanism by which a chemical causes toxicity. Therefore, the main goal of our research is to identify and validate key molecular toxicity pathways that are predictive of EE2- and FLX-induced apical responses in the model fish, *Pimephales promelas*. We will utilize sequence-by-synthesis-based whole transcriptome (RNASeq) and high-resolution mass-spectroscopy-based shotgun proteomics to characterize the molecular toxicity pathways and associate these with downstream biological responses of ecological and regulatory relevance in fathead minnow embryos exposed to graded concentrations of EE2 and FLX. We anticipate that this strategy will allow us to identify a set of signature toxicity pathways, including rare transcripts, that are predictive of EE2- and FLX-induced toxicity. This approach would facilitate the identification of key pathways and core genes involved in toxic responses, and therefore lead to the development an early life-stage gene expression assay that captures critical toxicity pathways for the prediction of apical outcomes of regulatory relevance beyond the two chemicals listed here. As this assay would take place prior to swim up, it would not be considered as a live animal test, hence, would address the need for alternative approaches in chemical screening. This study is part of the EcoToxChip project (@*ecotoxchip*).

Remediation and Restoration – Assessing and Measuring Effectiveness for Contaminated Sediment

WP061 A Field Evaluation of Performance Reference Compound (PRC) based Estimates of Equilibrium using Water Column Deployed Passive Samplers

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Low-Density polyethylene (LDPE) sheets are often used as passive samplers for aquatic environmental monitoring to measure the freely dissolved concentrations of hydrophobic organic contaminants (HOCs). HOCs that are freely dissolved in water (C_{free}) will partition into the LDPE until a thermodynamic equilibrium is achieved; that is, the HOC's chemical potential in the passive sampler is the same as its potential in the surrounding environment. However, achieving equilibrium for high molecular weight HOCs can take several months or even years. One way to evaluate the equilibrium status or estimate the uptake kinetics is by using performance reference compounds (PRCs). PRCs are often isotopically labeled versions of target compounds and are partitioned into the LDPE prior to deployment. Based on the fraction of each PRC lost during deployment, a sampling rate (R_s) or a fractional equilibrium (f_{eq}) can be determined for target HOCs, under the assumption that PRC desorption from the passive sampler occurs at the same rate as the unlabeled target HOCs. In this study, LDPE passive samplers were pre-loaded with six, ^{13}C-labelled PCBs as PRCs, and deployed in New Bedford Harbor, MA, USA. Triplicate samplers were collected after 30, 56, 99, and 129 day deployments. PRC-corrected C_{free} concentrations were estimated for 27 target PCBs (log K_{OW} ranging from 5.07 – 8.09) at each time point. Results allowed for calculation of desorption rates of PRCs as well as uptake rates for target HOCs and confirmed that kinetics are indeed isotropic for isomers. Results were fit to a traditional first order kinetic model, a sampling rate model, and a diffusion model to assess how well each predicted equilibrium C_{free}. Samplers at equilibrium showed agreement within 20%. However, for PCBs with slower kinetics, as the fractional equilibrium achieved decreased in magnitude, the C_{free} agreement between models and other time points also decreased. In general, results from the 30-day deployment illustrated the highest C_{free} for PCBs with a log K_{OW} greater than 6.5 or when a f_{eq} of 15% or less was achieved over the course of the deployment. These results provide a field-based evaluation of the usefulness of PRCs but also suggest caution should be used when correcting passive sampling data by a factor of 10 or more.

WP062 Evaluating Passive Sampler Performance Reference Compound (PRC) Modeling Methods in a Cross-Site Field Study

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Single phase polymeric passive samplers are increasingly used to estimate the dissolved concentration of hydrophobic organic contaminants in sediment pore water. To convert non-equilibrium passive sampler concentrations to more environmentally-relevant equilibrium pore water concentrations, performance reference compound (PRC) release rates are used to parameterize a mass transport model that is used to infer sampling kinetics. Two classes of mass transport models are typically applied: exponential (i.e., first-order) and diffusion-based. The objective of this work was to evaluate the two models in a cross-site field study with respect to performance, accuracy, and efficiency. Polyethylene passive samplers preloaded with 11 rare PCB congener PRCs were deployed at a number of field sites (three completed, three to six additional expected before November 2017) impacted with PCBs and/or PAHs. Estimated equilibrium polyethylene and pore water concentrations were calculated by both models and compared. The two models calculated practically identical estimates with respect to total PCB/PAH pore water concentration. The diffusion model resulted in total PCB/PAH equilibrium pore water concentration estimates that were a statistically insignificant (paired t-test, $p< 0.05$) 3% greater than the exponential model. While the difference between the two models is greater on a compound-by-compound basis, these differences were minor (< 20%) compared to other sources of uncertainty. These data suggest that, under the stagnant sediment conditions employed in this study, model choice is a relatively minor source of measurement uncertainty.

WP063 Laboratory, Field, and Analytical Procedures for Using Passive Sampling in the Evaluation of Contaminated Sediments: User's Manual overview

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Passive sampling is used for applications at contaminated sediment sites including performing assessments of contaminant bioavailability (i.e., freely dissolved concentration (C_{free})), conducting remedial investigations and feasibility studies, and assessing the potential for contaminant bioaccumulation. Previous research articles and documents have discussed many aspects of passive sampling however no definitive guidance on the laboratory, field and analytical procedures for using passive sampling at contaminated sediment sites has been provided. The document discussed in this presentation provides passive sampler users with the guidance necessary to apply the technology to evaluate contaminated sediments. Contaminants discussed include polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and the metals, cadmium, copper, nickel, lead and zinc. The document discusses different types of samplers used commonly in the United States, selection and use of performance reference compounds (PRCs), extraction and instrumental analysis of passive samplers, data analysis and quality assurance/quality control, and a list of passive sampling related references. The intent of the document is not to provide a series of standard operating procedures (SOPs) but rather to supply users with the information needed to develop their own SOPs. The document also includes the names of selected passive sampling experts that can address specific questions about laboratory, field and analytical procedures associated with passive sampling as well as selected laboratories currently providing passive sampling services. All of the information in this manual is intended to provide a sound foundation for passive sampler users to apply this valuable technology.

WP064 Using modeling to predict the performance of in situ treatment and assess performance of passive sampling

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Activated carbon is increasingly being considered for remediation of contaminated sediments sites. A common approach for application of the carbon is in a commercial formulation designed to settle to the surface of the sediment and to slowly be worked into the sediment over time by bioturbation or deposition of new sediments (e.g. in Aquagate and Sedimite). In this paper, simulation is used to predict the performance of activated carbon placed on the surface of sediments as an in situ treatment and to evaluate the performance of passive sampling which is commonly used to measure the effectiveness of the remedy approach. The predictions are compared to field measurements at a carbon treatment demonstration site near San Francisco, CA in which both Aquagate and Sedimite were allowed to settle to the surface of the sediments. CapSim, a robust model of contaminant fate and transport in the surficial sediments, is used to simulate carbon mixing over time into the surficial sediments coupled with the dynamics of uptake of PCBs onto activated carbon. The impact of bioturbation mixing rates and mass transfer limited PCB uptake is illustrated. Passive sampling using polydimethylsiloxane (PDMS) was used to measure performance of the in situ carbon treatment but the sampler uptake kinetics can be influenced by the nonlinear sorption of the activated carbon. In particular, performance reference compounds do not exhibit desorption rates equivalent to similar compound uptake rates in such an environment and simulations were also used to correct for these anomalies and improve the quality of the field passive sampling measurements of porewater concentration and associated bioavailability reduction. The field measurements of carbon mixing, porewater dynamics and bioavailability reduction were then compared to the predictive simulations using the CapSim model. The presentation provides both information as to how to correct for passive sampling anomalies in a nonlinear sorbing environment as well as an indication of how real world performance of surface placement of carbon may be limited by carbon mixing and uptake dynamics.

WP065 Fluxes of PCBs between Sediments and the Water Column from the New Bedford Harbor Superfund Site Using LDPE Passive Samplers in Laboratory Mesocosms

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Polyethylene passive samplers were deployed across the sediment water interface in experimental flow-through mesocosms consisting of sediment from New Bedford Harbor and artificial seawater. Passive sampling data was used to determine surficial sediment porewater and near bottom surface water concentrations of polychlorinated biphenyls (PCBs). A model of Fickian diffusion across a thin water side boundary at the sediment-water interface was used to calculate flux of PCBs due to molecular diffusion. Concentrations in six mesocosms in two sets of experiments (i.e., one with organisms present and one without) were used to calculate fluxes of selected PCB congeners (17, 52 and 110) from the sediment to the water column. Performance reference compounds (PRCs) were applied in passive samplers to correct for incomplete equilibration. Using diffusive water boundary layer thicknesses between 100 and 1000 µm, the calculated flux of selected PCBs across the sediment-water interface ranged from 5×10^{-3} to 2ng cm^{-2} d^{-1}. Positive values indicate flux from the sediment to the water column. Additional passive samplers suspended in the water column were used to determine PCB concentrations in the bulk water. Mass transfer due to diffusion across the sediment water interface was compared to mass transfer out of the mesocosm through overflow water. The mass transfers calculated through each route were in agreement within uncertainty as would be expected for a system at steady-state. These results indicate passive sampling can be applied to accurately calculate contaminant fluxes between contaminated sediments and relatively clean surface waters.

WP066 Identifying benthic recovery after sediment remediation in multistressed Hamilton Harbour, Lake Ontario

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Hamilton Harbour, at the western end of Lake Ontario, is an enclosed bay surrounded by a partially industrialized shoreline and urbanized watershed. It has been impacted by multiple stressors, including long-term exposure to wastes from steel industries, municipal wastewater discharges, and urban runoff. Sediments in much of the harbour are highly contaminated with metals and polycyclic aromatic hydrocarbons (PAHs), toxic to invertebrates in laboratory exposures, and sparsely populated by resident benthic invertebrates. In 2016 a major remediation project aimed at reducing metal and PAH masses and sediment toxicity began. The project, centred on the most contaminated section of the harbour, will involve dredging and confining the most contaminated sediment and covering the surrounding area bottom with a thin layer cap. Benthic conditions in the harbour have been assessed periodically since 1990 by examining sediment concentrations of contaminants, sediment toxicity to up to four invertebrate species, benthic macroinvertebrate community structure, and contaminant bioaccumulation in invertebrates. Determining ecological impairment and recovery is complicated by a lack of suitable reference sites and effects of other stressors that will not be mitigated by the remediation project, including seasonal hypoxia at depths >7 m, physical disturbance of sediment by ships, urban runoff, and municipal waste water discharges. Benchmarks for recovery were developed for each assessment component based on comparisons to best available reference conditions and adverse effect-associated criteria. Reference conditions were defined as either (a) minimally disturbed sites in the harbour (littoral and profundal zone separately) where stressors are lowest or (b) predictions for low- or no stressor-conditions from ecotoxicological models that relate benthic community structure to habitat and stressor variables. Adverse effect-associated criteria include sediment quality guidelines, toxicity >20% of that for reference conditions, tissue residue guidelines, and *a priori* selected biocriteria.

WP067 Buried, but not forgotten: Activated carbon caps can sustain their remediation efficiency when covered by dynamic sediment movement

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Thin layer capping of contaminated sediments with activated carbon (AC) is a novel and promising approach to *in-situ* remediation. The capability of AC to strongly bind organic pollutants can greatly reduce their bioavailability and sediment to water flux. However, this remediation method faces several challenges to its long-term efficiency. A problem that can occur especially where waterbodies are only treated partially is the redistribution of sediment from adjacent sites on top of the AC layer. This was for example a great issue in the first field-trial of AC capping in Finland. The large surface area and the shallow depth of the treated, PCB-contaminated Lake Kernaalanjärvi caused frequent sediment movements. The applied AC cap was rapidly covered with contaminated sediment, leading to a low remediation success. To investigate the possibility of the AC caps to retain their remediation efficiency under these dynamic conditions, the scenario from the field test was replicated under more controlled laboratory conditions: PCB-contaminated sediment was amended with an AC cap (1.2 kg/m^2), which was topped with a second layer of contaminated sediment (thicknesses ranging from >1 - 40 mm). *Lumbriculus variegatus* and *Chironomus riparius* were exposed and their accumulation of PCBs was used to assess the remediation efficiency. In addition, the adverse effects of the sorbent material itself were measured (organism growth). With the deeper dwelling *L. variegatus*, the AC cap was mixed rapidly into the sediment layers, leading to a significantly reduced PCB bioaccumulation (53-71 % lower than in unamended sediment). Only with large amounts of sediment (> 20 mm) atop the AC layer, the reduction in PCB bioaccumulation was no longer significant. With the shallow dwelling *C. riparius* on the other hand, the effects of sediment deposition on remediation efficiency were much more pronounced. The reduction in PCB uptake was no longer significant with as little as 5 mm sediment covering the AC cap. The adverse effects of the AC material itself correlated with the measured remediation efficiency: the reduced PCB uptake was accompanied by reductions in the organisms' growth. In general, this study shows that AC caps can retain their effectiveness, even when covered with contaminated sediment. The remediation success, however, might strongly depend on the benthic community health and structure – which in turn can be negatively affected by the adverse effects of the AC itself.

WP068 Monitoring the Hudson River Post-Remediation: When Can We Expect to Achieve Remedial Goals and How Do We Measure it? The 2017 Five-Year Review

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The investigation and remediation of the Hudson River PCBs Site (NY) spans a period of nearly 30 years. With the completion of active remediation of the Hudson in 2016 and the extensive historical and post-remediation monitoring programs, the site provides the opportunity to track conditions through time and collect sufficient data to evaluate remedy effectiveness. The Hudson remediation, involving the removal of 2.7 Myd^3 of sediment followed by backfilling, restoration and replanting, was completed in Oct. 2016, initiating a period of monitored natural attenuation across more than 150 river miles, as mandated by the 2002

Record of Decision. The monitoring of PCBs in water and fish throughout the Hudson began many years prior to the remediation, and is slated to continue for decades as PCB levels in fish tissue respond to the active remediation and then continue to decline toward the final remedial action objectives. Additionally, EPA added a long term surface sediment monitoring program and routine monitoring of Be-7 bearing sediments. Together these monitoring elements track not only the remedial endpoint (PCB levels in fish) but also PCB levels in the matrices responsible for fish exposure across nearly all of the impacted areas post-remediation. EPA recently completed its second five-year review report for the site to assess the status of the remedy regarding protectiveness of public health and the environment. The most recent monitoring data suggest that PCB levels in fish tissue have begun to recover from dredging impacts and are generally declining throughout the Upper Hudson. Water column data have responded in a similar manner. Finally, the initial post-dredge long term sediment monitoring results are consistent with a continued declining trend suggested by the historical data. EPA is using these data in combination with its earlier modeling work to assess recovery including approximate times to reach milestones and remedial goals in the Upper Hudson fish. Fish trends in portions of the Lower Hudson suggest that this region of the river may be recovering more slowly than anticipated, which supports the need for further evaluation of the influence of remedial work in the Upper Hudson on the Lower Hudson. Also several factors related to project implementation may influence estimates to achieve remedial milestones. This presentation will summarize the results of the five-year review, including EPA's estimates for attainment of the remedial objectives.

WP069 Investigating the efficiency of remediation of TCE contaminated groundwater using permeable reactive barrier

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One of the most frequent contaminants in groundwater is trichloroethylene (TCE), a volatile aliphatic organic compound which can cause health problems such as cancer, liver and kidney damages and neurological problems. An aquifer in Maryland, is contaminated with high levels of TCE. In 2013, a permeable reactive barrier (PRB) was installed across the flow direction between the landfill (source of contamination) and the nearby wetland. PRB was made of mulch, compost, and sand which can provide a good condition for the microbial community to grow and form a consortium capable of degrading TCE to ethane. TCE can be reductively dechlorinated by some microorganisms. Regular samplings of the groundwater shows that the PRB was effective degrading TCE into less chlorinated compounds. Results from the latest samplings, three years after installation of the wall, indicate TCE levels have decreased significantly, down gradient the PRB (600 ppb up gradient to below 1 ppb, down gradient the wall). Also, with decrease in TCE levels, TCE degradation products have increased. A similar behavior was observed for the well closest to the biowall, indicating presence of a "dehalo" effect. Little ethene production has been observed down gradient the PRB, despite the increase in vinyl chloride (VC) concentration (from not detectable up gradient the PRB to 19 ppb down gradient the PRB). This observation indicates that the microbial community present at the site, are not efficiently degrading VC. In this study, we are going to focus on VC degradation processes. To obtain more information on microbial community present in the PRB and find the species that are potentially able to degrade VC at the dehalo zone, sequencing and the enrichment of microbial consortia of soil will be conducted. Batch reactors with different amendments (such as organic carbon and consortia of TCE and VC degrading microorganism) will be set up to find the best treatment for VC degradation.

WP070 An Independent Evaluation of the Hudson River PCB Dredging Program

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In February 2002, the General Electric Company (GE) was ordered by the U.S. Environmental Protection Agency (EPA) to conduct targeted dredging of PCB-contaminated sediment in a 40-mile stretch of the Upper Hudson River between Fort Edward and Waterford, NY. GE performed dredging of the Upper Hudson in two phases, beginning in May 2009 and ending in October 2015. Following completion of dredging operations, the Hudson River Foundation convened an expert panel to evaluate the effectiveness of the dredging program on the Upper and Lower Hudson. Based on water column and fish monitoring data and other information that were available through December 2016, the panel concluded: (i) the dredging program met mass removal targets for PCB-contaminated sediments, (ii) the dredging program was effective in reducing PCB concentrations in fish from Thompson Island Pool, (iii) post-dredging PCB concentrations in fish downstream of Thompson Island Pool showed mixed results, (iv) the reduction in Tri+ PCB loads to the Lower Hudson during the 2016 post-dredging period were in part due to below-average flows in the river, (v) water column, sediment and fish in the Lower Hudson below Albany are showing slow responses to the Upper Hudson dredging program due to the complexities of sediment transport in the Lower Hudson, and (vi) additional years of natural attenuation will be required to reduce PCB concentrations in fish throughout the Upper and Lower Hudson to acceptable levels. Modifications to the post-dredging monitoring program and continued evaluation of the next few years of monitoring data are therefore recommended to assess if natural attenuation will be sufficient in reducing PCB concentrations in fish in a reasonable time frame or if additional remedial actions will be required.

WP071 Conceptual design of adaptive and integrated approaches to monitoring of post-remediation recovery: Examples from Great Lakes Areas of Concern

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Because of the complexity of natural and disturbed aquatic ecosystems, there are always unanticipated outcomes that result from large-scale remediation at contaminated sediment sites. A thoughtful and comprehensive site model that is regularly revised with new information as remediation and recovery unfold is essential for long-term success of such projects. Using examples from Great Lakes Areas of Concern, we will illustrate how intentional adaptive management approaches can lead to better decision-making during and after remediation. Components of a robust restoration program include clear and realistic goals, an accessible and flexible data management and communications subsystem, comprehensive baseline data, a well-designed and adaptive monitoring program, a team of committed scientific and technical advisors, and a process governance backbone with clearly defined roles and responsibilities. Targets of monitoring at Great Lakes sites typically include status and trends of sediment and water quality and dynamics, benthic community composition, fish community composition and health parameters, and human satisfaction with the state of beneficial uses in the system. The governance structure must include processes for assimilating new information on a variety of timescales (days to decades), and mechanisms for changing course and bringing in new resources when approaches are not working, or where natural or human-induced disturbances (e.g., spills, new invasive species, floods, innovation) alter the trajectory of system recovery.

WP072 Long-term stability and efficacy of historic activated carbon (AC) deployments at contaminated sediment remediation sites

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Aquatic sediments form the ultimate repositories of past and ongoing discharges of hydrophobic organic compounds (HOCs) such as PCBs. Sediment HOCs can be taken up by pelagic or benthic organisms through ingestion and dermal absorption, and subsequently passed on to higher organisms and humans. For both of these pathways, the uptake exposure depends on the bioavailability of contaminants in sediment. Work in the last two decades has demonstrated that black carbonaceous particles in sediments such as soot, coal, and charcoal very strongly bind HOCs, and their presence in sediments (both natural and anthropogenic) reduces exposure. The long-term efficacy of AC as an approach to in situ remediation has been a recurring and central question raised in discussions among regulators and the parties conducting sediment clean-ups. The objective of this study is to assess the long-term (6-10 yr) performance of AC at multiple sites and determine the relative importance of physical processes to long-term efficacy. This will be accomplished by determining the mass of AC present and its vertical distribution within the sediment following the initial introduction and determining if the AC still retains its functional efficacy (PCB sorption, bioavailability reduction). We resampled pilot scale remedy applications at the Grasse River (NY) and Canal Creek (MD) which are 10 and 6 years post placement, respectively. Our samples recovered AC at all treated sites, however the depth varied based on site specific variation. The contrasting data sets demonstrate the importance of physical processes, given that one site is a slow flowing river and the other is a vegetated marsh under tidal influence. Bioaccumulation testing and analytical chemistry data will characterize the long term efficacy of AC in situ remediation. These data which will further develop lines of evidence to be incorporated into future remedial actions involving activated carbon.

WP073 Value in Refining Risk Assessments for Remedial Design

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Risk assessment is a standard tool employed during remedial investigation of contaminated sites. Conservative and generic assumptions are often used to simplify the risk assessment process and to ensure that risks are not underestimated and health protectiveness is achieved. However, such assumptions may not be wholly appropriate and can result in greatly overestimating risk for a given site. Risk results are then carried forward into the feasibility study (FS) and in many cases the assumptions are never refined or reviewed for their applicability; remedial footprints are then estimated using these initial assumptions. Furthermore, site conditions can change by the time the remedy is implemented, also making the original assumptions no longer valid. An example is presented where the initial risk assessment was based on conservative and general assumptions using data more than 20 years old. Potential risk to invertebrate-eating waterbirds (e.g. stilts and ducks) from exposure to polychlorinated biphenyls (PCBs) in sediment were estimated using a sediment-to-fish bioaccumulation factor (BAF) as a surrogate for an invertebrate BAF. Fish BAFs for lipophilic compounds like PCBs tend to be higher because lipid content in fish tends to be higher than in invertebrates, which subsequently drives bioaccumulation potential, and can result in an overestimate of exposure to birds and their risks. Additionally, appropriate foraging habitats were not accounted for in the site use factors (SUFs), which also contributed to overestimation of risk for receptors (e.g. stilts prefer shallow water areas with depths of less than 9 inches). As part of the pre-remedial design, the risk assessment was refined by incorporating refined, site-specific exposure conditions, applicable receptors, and bioaccumulation. Recent sediment and invertebrate samples were collected to estimate a site-specific invertebrate BAF. Risks estimated using recent sediment data, site-specific invertebrate BSAF, and appropriate SUFs indicated no unacceptable risk to waterbirds and the preliminary remediation goal was approximately 10 times greater than originally derived. A sensitivity analysis was conducted using a range of invertebrate BAFs (epifauna vs macroinfauna) and SUFs. To manage the potential for change, flexibility should be written into the FS, proposed plan, and record-of-decision to allow for necessary updates in the risk assessment which can be incorporated during design phases.

WP074 Assessment of the physical mechanisms behind sediment transport and contaminant distribution in the Manistique River

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The Manistique River Area of Concern (AOC) covers the last 1.7 miles of the river in Manistique, MI. This area has a legacy of contamination from industrial waste, including PCBs in sediments and woody debris (e.g. sawdust) within the harbor, river channel, and nearby beaches. As in most AOCs, sediment transport in Manistique River (MR) is subjected to flood flows and meteorologically-induced water level oscillations. These lake oscillations have periods between several minutes (high-frequency water level oscillations) and a few hours (e.g. seiche). After investigation into the physical drivers behind resuspension and transport in the Manistique River, we reveal transport of contaminated sediments inside the harbor is caused by high-frequency oscillations (e.g. meteotsunami), seiches, and flood flows. Bottom shear stresses induced by these driving forces can exceed the critical condition to resuspend bottom sediments even in a deep water environment. The meteorologically-driven water level oscillations can temporarily reverse flow in the MR and transport contaminated sediments upstream to previously cleaned areas. Results indicate that while flood flows are the main cause of sediment transport in the main stream, meteorologically-driven oscillations are the primary cause of resuspension and flushing of contaminated sediments, which are usually located outside the main stream. Overall the outcomes of this study aid in addressing the sustainable remediation of a river-estuary AOC of the Great Lakes.

WP075 Wheat Straw Biochar and Wheat Straw Hydrochar for the Removal of Heavy Metals in Wastewater

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In recent years, wastewater reclamation is gaining importance as a freshwater conservation technique. The presence of inorganic contaminants, such as heavy metals in wastewater, could reduce the quality of agricultural soils when irrigated with such water. Biochar, a product of biomass pyrolysis, can remove heavy metals from polluted aqueous solutions. However, hydrochar, the product of hydrothermal carbonization of biomass, has attracted researchers to investigate its role in the removal of heavy metals in wastewater. Due to differences in their carbon content, specific surface area, and pore volume, hydrochar and biochar from the same feedstock might function differently as a biosorbent for wastewater contaminants. Therefore, the aim of this study is to compare the sorption capacity of biochar and hydrochar, produced from the same feedstock (Wheat straw). The heavy metals of interest are commonly found in untreated wastewater, namely Pb, Zn, Cd, Cu, Cr and Fe. Batch equilibrium experiments have been conducted in triplicate for lead in aqueous solutions. The experimental results fitted well to the Langmuir mode, with sorption capacity of 68,960 mg/kg and 9,617 mg/kg for biochar and hydrochar, respectively. This suggests that wheat straw biochar has a higher sorption capacity for lead, compared to hydrochar. This could be explained by the lower pH of the hydrochar solution which could affect the ion exchange capacity of hydrochar's sorption sites. Further experiments to determine the sorption capacity of wheat straw biochar and hydrochar for the rest of heavy metals are in progress.

WP076 Developing Bio-remediation Technology Using Pseudomonas putida and Poplar for Restoring the Petroleum Contaminated Sites

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Source of energy for a daily life in Indonesia and USA mostly still derived from petroleum and gas sources. Despite the high demand on petroleum and gas, the mining process itself can be harmful for the environment and public health. Approximately there are more than 750 chemicals that were used for mining process and many of those chemicals are toxic or they behave as an endocrine disruption for human and animals. Due to the number of operating petroleum mining and potential of shale gas exploitation, it is necessary to prepare the most cost-effective and environmentally friendly restoration strategies. Bioremediation is an emerging cost-effective approach to restore the sites contaminated with petroleum hydrocarbon pollutants. The proposed approach synergistically integrates the microbial degrader Pseudomonas putida with 20 different clones of poplar trees. This study aim to investigate the effectiveness of the P. putida bioremediation approach in removing the pollutants and restoring the soil health of the petroleum contaminated sites. The concentrations of the pollutants and its degradation products will be quantified using SPME (solid-phase micro extraction) followed by GCMS (gas chromatography mass spectrometry) analysis and degradation kinetics will be determined. The persistence of the introduced degraders into the rhizospheres will be monitored by TaqMan real-time quantitative PCR. The soil health and soil microbial community will be characterized using metagenomics deep-sequencing. The outcomes of this research expected to be applied on post petroleum mining site and help to protect the public health from pollutant exposure in Indonesia, USA and other oil producing countries.

Approaches for Deciphering Multiple Stressors in Aquatic Environments

WP077 A multibiomarker analysis of pollutant effects on Atlantic stingray populations in Florida's St. Johns River

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The goal of this study was to examine the potential health effects of organochlorine (OC) and polycyclic aromatic hydrocarbon (PAH) exposure on Atlantic stingray (*Dasyatis sabina*) populations in Florida's St. Johns River (SJR). Special emphasis was placed on identifying OC- and/or PAH-related effects in stingrays from areas of the lower (LSJR) and middle (MSJR) basins that have been shown to possess elevated levels of these compounds, as well as characterizing baseline levels of pollutant exposure in the LSJR shipping channel, which may be subjected to dredging in the near future, potentially resuspending and redistributing contaminated sediments and increasing pollutant-associated effects. To accomplish this, we measured biomarker levels in stingrays collected from contaminated and reference sites. We specifically examined the biomarkers cytochrome P4501a1 (CYP1a1), a phase I detoxification enzyme; glutathione-S-transferase (GST), a phase II detoxification enzyme; uridine 5'-diphosphate glucuronosyltransferase (UGT), a phase II detoxification enzyme; fluorescent aromatic compounds (FACs), PAH bile metabolites; and lipid peroxidation (LPO), cell membrane damage. Data was analyzed using the Integrated Biomarker Response (IBR), which synthesizes data from multiple biomarkers into a single number, allowing for a comparison of samples by site. IBR values of individuals collected between 2014 and 2016 were compared by site. Detoxification enzyme activity and LPO values from MSJR individuals collected between 2002 and 2005 were compared to biomarker levels of MSJR individuals collected between 2014 and 2016. The data suggest that IBR values in stingrays from a reference site near Brunswick, Georgia, and the MSJR were significantly higher than those from Florida estuaries, including the LSJR. This indicates that residing in the MSJR is detrimental to stingray health, but residing in the LSJR is not. Two of the three lakes evaluated in the MSJR, Lake George and Lake Monroe, had GST and LPO values, respectively, that indicate reduced contaminant input over time. In contrast, CYP and UGT values from Lake Jesup of the MSJR suggest elevated contaminant input over time. This study has developed a baseline for biomarker levels in the LSJR, allowing for the identification of dredging-induced changes to the system, and has identified temporal changes in biomarker levels from the MSJR.

WITHDRAWN

WP078 Assessment of mixed chemical contamination in water and sediment samples from wadeable streams in the piedmont region of the Southeast United States

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Complex contaminant mixtures and associated aquatic-health effects are growing concerns. Replicate (n = 3-10) water samples were collected from 54 small Piedmont (USA) streams over 10 weeks in 2014 and analyzed for 489 organic analytes (470 unique chemicals), including 110 pharmaceuticals and degradates, 226 pesticides and degradates, 84 volatile organic chemicals, and 69 organic waste indicator compounds. Of these, 264 (56%) were detected at least once across all sites. Approximately 70% of the detected water contaminants were designed-bioactive organic chemicals (e.g., pharmaceuticals, pesticides). Cumulative detections and concentrations ranged up to more than 150 compounds (median greater than 80) and 160 $\mu g\ L^{-1}$ (median greater than 10 $\mu g\ L^{-1}$) respectively, and correlated significantly with wastewater discharge and watershed development metrics. Bed-sediment samples (n = 1) from each stream also were analyzed for 117 current-use pesticides, 55 halogenated-organic chemicals, and 38 polycyclic aromatic hydrocarbon compounds. Due to multiple modes of action, high bioactivity, biorecalcitrance, and direct environment application (pesticides), frequent detection of designed-bioactive organics (range up to more than 100; median greater than 40 per site) at cumulative concentrations up to $\mu g\ L^{-1}$ raise concerns for sub-lethal effects to sensitive aquatic species and lifecycle stages. The possible effects of the complex chemical exposures observed in this study were computationally predicted using knowledgebase-leveraging tools.

WP079 Assessment of nutrients in streams of the Piedmont area of the Southeastern United States

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During 2014, the U.S. Geological Survey National Water-Quality Assessment Program assessed stream quality across the Piedmont region in the southeastern United States. The goal of the Southeast Stream Quality Assessment is to characterize multiple water-quality factors that are stressors to aquatic life – contaminants, nutrients, sediment, and streamflow alteration – and the relation of these stressors to ecological conditions in streams throughout the region. Findings from the assessment will provide communities and policymakers with information about which human and environmental factors are most critical in controlling stream quality, and, thus, provide insight about possible approaches to protect and improve stream quality. The targeted design of the assessment identified and selected sites that reflected a range in the amount of urbanization and streamflow alteration. Seventy-eight multi-stressor sites were selected and sampled across the region for as many as 10 weeks during April, May, and June 2014 for contaminants, nutrients, and sediment. This water-quality "index" period culminated with an ecological survey at all sites. Fifty-nine sites were on streams in watersheds with varying degrees of urban land use, including 17 of the 59 with permitted wastewater discharge. Five sites were on streams with numerous confined feeding operations and 13 were reference sites with little or no development in their watersheds. This presentation will provide preliminary findings from this study and focus on spatial distribution of nutrients and related watershed characteristics of selected streams that drained a gradient of urbanized, animal-feeding-operation-dominated, and reference conditions. The data were assessed regionally and by 5 urban center.

WITHDRAWN

WP080 Optimizing the In Situ Toxicity Identification Evaluation (iTIE) Technology as a Diagnostic Tool to Establish Stressor-Causality Linkages

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It is difficult to assess the toxicity of a single stressor and establish a strong stressor–causality link when multiple stressors coexist. The USEPA's Toxicity identification evaluation (TIE) methodology uses a series of chemical and physical manipulations to fractionate compounds within a matrix and systematically identify potential toxicants. The TIE provides useful information but lacks ecological realism as it is subject to laboratory-related artifacts. The in situ TIE (iTIE) technology (Burton & Nordstrom 2004), is a biological-chemical fractionation system that systematically identifies causes of toxicity. The iTIE has undergone a number of iterations to increase the robustness and reliability of the novel technology. The 2017 prototype consists of a rectangular unit capable of housing an array of iTIE units. Each unit is equipped with an organism exposure chamber, a smaller chamber filled with a resin absorbent to fractionate porewater, surface water or effluent passing through the organism chamber, and a connection to a water collection container. A secondary containment unit is used to house the electronic controller along with other components which connects to the primary iTIE housing array. The unit consists of a water proof housing containing a programmable circuit board, pumping units, and rechargeable battery. The iTIE system was deployed to a depth of 3 meters and evaluated in streams and marine harbors. The system assisted weight-of-evidence studies to assess stream impairments, wastewater effluents, porewaters and groundwater-surface water interactions using multiple species. Chemical analyses of water and iTIE chemical sorptive resins confirmed lethal to sublethal responses. This latest iTIE prototype provides a robust technology that improves stressor-causality linkages.

WP081 Use of reference toxicant challenges for studying multiple stressor impacts

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Much of the pharmaceutical research to date has been on single compounds and their potential toxicity to aquatic organisms. Although single compound exposures are important to understanding the cause and effects of pharmaceuticals on non-target organisms, they don't represent the real environment in which fish would be exposed. In surface waters, wild fish are exposed to numerous compounds over multiple generations with many different stressors and modifying factors. A short-term reproduction study was completed using adult American flagfish (*Jordanella floridae*) that were exposed to ibuprofen, naproxen, and 17 α-ethinylestradiol alone, and in mixtures. Offspring were collected and used in a copper reference toxicant challenge. The challenge was completed to assess if their sensitivity would alter based on prior life history. There was a slight change in sensitivity noted during the challenges, however, the change was noted for both the control and prior exposure fish. The methodology of testing subsequent exposures on offspring as a secondary stressor has not been well studied. This research aims to contribute to a better understanding of the impact of multiple factors including single and mixture pharmaceutical exposures and subsequent toxicant challenges which fish may encounter in the environment.

WP082 Visualizing results of complex multi-stressor environmental assessments online

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How can scientists effectively communicate results and share data for large multi-stressor and multi-disciplinary studies? The team of scientists working on the U.S. Geological Survey (USGS) Regional Stream Quality Assessment (RSQA) has developed a Web site to provide RSQA data and results to scientists, government agencies, and the interested public (https://txpub.usgs.gov/RSQA/). During 2013–17, RSQA scientists characterized water quality, sediment quality, and habitat to determine the individual and combined effects of hundreds of potential chemical and physical stressors on biological communities in streams. Water, sediment, habitat, and biological condition of 80 to 100 small streams were assessed in each of five regions of the United States: the Midwest, the Southeast, the Pacific Northwest, the Northeast, and Coastal California. More than 2 million individual chemical results were generated, as well as extensive data on streamflow and temperature, stream habitat, geospatial characteristics of watersheds, and biological communities in the streams. These data are being interpreted using statistical models based on metrics that attempt to condense the raw data into meaningful indicators of ecological stress and community responses. The RSQA Web site makes it possible to visualize key metrics of regional and national results; time-series data for individual constituents at a site are provided, as well as site-specific results in a "report card" that summarizes major water- and sediment-quality constituents, habitat characteristics, and ecological metrics. Data can be downloaded using a menu-driven approach that allows the user to filter by region, state, sample medium, constituent group, and individual constituent. Links to publications also are provided. The goal of the Web site, powered by the USGS Texas Water Science Center, is to create a user-friendly environment where it is easy to understand and access the wealth of multi-stressor information available from the RSQA.

Unique Laboratory and Field-Based Methods to Address Complex Environmental Issues

WP083 Assessing the Trophic Transfer of Selenomethionine to an Amphipod (Hyalella azteca) Through A Diet of Field-Collected Microorganism Communities

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The mobilization and contamination of selenium (Se) into environmental systems is of significant global concern. A variety of natural and anthropogenic sources and site-specific differences in biogeochemistry are the source for different forms and concentrations of Se in aquatic environments (eg. selenate [SeO4?²] and selenite [SeO3?²]). These inorganic forms of Se are efficiently assimilated, biotransformed, and bioaccumulated by aquatic microorganisms into organoselenium compounds, which are transferred to higher trophic levels via dietary pathways. The present study aims to quantify the trophic transfer factors of selenomethionine to a primary consumer through the biotransformation of inorganic oxyanion Se forms (selenate and selenite) by field-collected communities of microorganisms. Biofilm samplers were placed in the epilimnetic zone of uncontaminated lakes showing a variety of basic water chemistries in northern Saskatchewan. They were allowed to accumulate natural communities of periphytic microorganisms representative of these lake habitats. DNA samples were collected from each biofilm sample for microscopic and metagenomic characterization of the biofilm communities. Samples were exposed in the laboratory to aqueous concentrations of selenite and selenate respectively, at concentrations of 0, 5 and 25 µg Se/L, and uptake and biotransformation by the biofilm to selenomethionine was quantified. The amphipod *Hyalella azteca*, a primary consumer characteristic of Canadian freshwater ecosystems, grazed on the spiked biofilm communities with the intent to determine trophic transfer efficiencies of Se as a function of microorganism community structure. This research will serve to assist in improving environmental risk assessment strategies for the release of Se into aquatic environments.

WP084 Importance and efficiency of ascorbic acid in fish embryo as defensive substance to chemical exposure

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Ascorbic acid (AsA) is one of essential substances for the survival of organisms. At first, when a collagen is polymerized in the body, AsA is absolutely needed. If AsA is shortage, the defective collagen is composed. AsA can also work as an intense antioxidant to the reactive oxygen species and protect an organism from the oxidative stresses derived from several kinds of stressors including toxicant. There are many additional efficiencies of AsA. In our preliminary study, when medaka (*Oryzias latipes*) embryos were exposed to oxygenated polycyclic aromatic hydrocarbons (oxyPAHs), we observed the remarkable reduction of AsA. Additionally, the larvae with morphological abnormalities hatched from the exposed embryos. Therefore, we hypothesized that the abnormalities was possibly caused by the shortage of AsA, because AsA should be used as the antioxidant to oxidative stresses induced by the exposure of oxyPAHs. In the present study, we obtained the embryos with the shortage of AsA, spawned from fish continuously fed AsA-free artificial food. Then, these embryos were reared until hatching, and examined the effects caused from AsA shortage, such as the abnormalities during development and the hatching rate of larvae with morphological abnormalities. We also examined whether the adverse effects are emphasized depending on the shortage of AsA, when embryos are exposed to chemicals. We used medaka as test fish, because medaka can produce little AsA in the body, and absolutely requires to take AsA from food. We prepared the artificial food without the fish meat. The food for control group was contained 300 mg/kg AsA. AsA-free food was also prepared using same component materials with control group, except for AsA. Parent medaka were continuously fed these artificial food for a month, and the spawned embryos were sampled and reared until hatching. As a result, the egg membrane in AsA-free group caused some abnormalities compared with control group. In addition, the hatching larvae induced the curvature of vertebra and other abnormalities. Then, we exposed 7,12-benzo(a)anthracenequinone (BaQ) to embryos spawned by parent fish fed control or AsA-free foods. The exposure resulted to be lower median lethal concentration in AsA-free group than that in control group. This result suggests that the oxidative stresses derived from exposure of BaQ intensely developed in AsA-free group compared with control.

WP085 Meta-analysis of PCB toxicity data in fish for determination of data-driven tissue concentration-based thresholds of effects

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Polychlorinated biphenyls (PCBs) are a class of organic compounds with historic industrial use and a legacy of environmental contamination. Despite active use and production terminating in the 1970s, PCBs persist in the environment and still have the potential to impact aquatic life. A huge volume of information has been generated assessing the environmental impacts of PCBs. While many federal, state, and local programs have assessed PCBs, the specific federal regulations mandate that the Department of Interior, through the Natural Resource Damage Assessment and Restoration (NRDAR) program, determine environmental injury in areas where PCBs have entered the environment. Our objective was to develop a broad, transparent, data-driven evaluation of PCB impacts in the context of NRDAR, starting with fish. For this assessment, data from the literature were selected based on specified criteria. Criteria were developed to account for the requirements for NRDAR assessments and known chemical and biological interactions of PCBs and fish. To provide a model for further assessments, the scope of this meta-analysis was limited to PCB-related mortality, growth, and reproductive (MGR) responses, effect endpoints that tie most directly to injury. For each type of effect the LOAEC (lowest observable adverse effect concentration) was collected along with a percent effect. The LOAEC, was in this case a tissue concentration, based on the bioaccumulative nature of PCBs. The LOAEC was selected as it represented a direct measure of effects, thus providing better translation from effect to injury. The MGR datasets were included only laboratory data, but responses were supported by field-observed effects. For the M, G, and R datasets, concentration-response threshold regressions were developed. These provided a means to determine the degree of adverse response expected across the broad range of PCB tissue concentrations. For example, at a PCB tissue concentration of 10 µg/g, the regression would estimate a 29% increase in mortality, a 39% reduction in growth, and 50% inhibition of reproduction. This regression approach also allowed for the development of data-driven uncertainty assessments. Data generated from MGR endpoints were combined to determine an overall effect threshold for fish and PCBs. This work provides a comprehensive assessment of NRDAR-defined injury to fish caused by PCBs utilizing a data-inclusive approach.

WP086 Overview of Public Comments Received on the USEPA Draft Aquatic Life Conductivity Methods Document

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The USEPA released the *Draft Field-Based Methods for Developing Aquatic Life Criteria for Specific Conductivity* for public comment on December 23, 2016. Once final, states and authorized tribes may use these methods to derive field-based ecoregional ambient Aquatic Life Ambient Water Quality Criteria for specific conductivity (SC) in flowing waters. The methods document provides flexible approaches for developing science-based SC criteria that reflect ecoregional or state specific factors. The public comment period was extended for an additional 62 days from February 21, 2017 to April 24, 2017 due to stakeholder requests. A total of 67 sets of comments were received. Comments included responses from a variety of stakeholders including those from industry, states, publicly owned treatment works, and private citizens. Public comments were arranged into major categories in order to facilitate the development of responses. This poster presents the major categories of submitted public comments which will be considered in developing the final version of *Field-Based Methods for Developing Aquatic Life Criteria for Specific Conductivity*. These are the authors views and do not necessarily represent views or policies of USEPA.

WP087 Polymorphism Comparison of Tumor Suppressor Gene, P53, from Different Organs of Wild Gulf Menhaden, Brevoortia patronus

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British Petroleum Deep Water Horizon Oil spill in the Gulf of Mexico (GOM) caused detrimental effects to wildlife including marine fish populations. The fish were exposed to crude oil which contains polycyclic aromatic hydrocarbons (PAHs). Some types of PAHs are known to be long-lasting carcinogens that damage DNA including key regulatory genes in various species. Menhaden are oily and contain a high amount of omega-3 fat subsequently giving them a higher chance to incorporate and retain these lipophilic contaminants. In addition, their risk of exposure is enhanced by their unique filtering feeding lifestyle. Prior work has shown high levels of PAH-like compounds in menhaden collected along the GOM and NJ coastline. In order to investigate PAH damage to critical DNA targets, effects on the proto-oncogene, P53, were measured in Gulf Menhaden (*Brevoortia patronus*) from the Vermilion and Barataria Bay areas of the GOM. The DNA binding region of the P53 gene was amplified by PCR using genomic DNA from various organs. Single stranded Conformational Polymorphism (SSCP) was also utilized to discover DNA polymorphisms possibly associated with P53 mutations generated by PAH exposure. P53 was utilized due to its function as a tumor suppressor gene, which is essential in regulation of the cell cycle. P53 normally functions to inhibit cell growth and division when excessive DNA damage is present. When mutated, uncontrolled cell growth is exhibited which is a hallmark of various cancers. For this reason, P53 polymorphisms were chosen as the model biomarker of environmental exposure in aquatic species. The goal of the present work was to determine if different organs in the fish may be more effected than others. Skeletal muscle, gill, liver, gonad and heart were evaluated. Preliminary results showed similar sequences for the different organs of the same fish.

WP088 Preliminary Evaluation of Improved Toxicity Testing Methods for Episodic Discharges

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Whole Effluent Toxicity (WET) tests for monitoring continuous point source discharges are highly standardized, and typically include static (or one or more test solution renewals) exposure of an end-of-pipe sample over 4-7 days, depending on the test species. However, ephemeral discharges, such as stormwater runoff, can be time-and magnitude-varying. Conservative runoff duration estimates over a given 96-hour period during rain events in San Diego, CA, USA, for example, are on the order of 12 to 25 hrs (75^{th} and 95^{th} percentile, respectively), based on datasets covering at least 20-yrs. Therefore, in our estimation, standard WET test methods may require modifications to improve relevance with respect to response by ecological receptors and regulatory compliance. We are critically evaluating the feasibility of using site-specific rainfall runoff duration, and a multitude of additional measures associated with time-varying discharges, to derive more environmentally relevant toxicity exposure regimes for stormwater compliance. Factors considered include (1) runoff duration over relevant time periods (e.g. the typical 96-hr static toxicity exposure); (2) conservative considerations with respect to life stage sensitivity; (3) effects and potential application of repeated pulses; (4) the potential for latent effects following standard exposure; and (5) empirical validation of the above using in situ toxicity testing in the receiving environment. Preliminary results associated with this work will include chronic toxicity tests with purple sea urchin (*Strongylocentrotus purpuratus*) embryos and acute toxicity tests with the mysid shrimp (*Americamysis bahia*), based on standard USEPA protocols, and slightly modified, to evaluate the relative sensitivity of these species to common stormwater-associated toxicants, including copper and zinc under static (96-hr continuous exposure) and pulsed exposures (time varying toxicant exposure, followed by transfer to uncontaminated seawater for the remainder of the 96-hr exposure period). To date, results with these species and endpoints suggest pulsed exposure EC50 or LC50 values up to two orders of magnitude greater (i.e. less sensitive) than current recommended continuous approaches for these tests. This presentation will summarize results to date, and future plans to assess the proposed WET modifications within National Pollutant Discharge Elimination System (NPDES) requirements for episodic discharges such as stormwater.

WP089 Using laboratory experimental streams to assess influences of hyporheic upwelling on the toxicity of zinc contaminated sediments

A. Harrison, G. Burton, University of Michigan / School of Natural Resources & Environment

Despite the importance of groundwater-surface water interactions for aquatic habitat, we have limited understanding of how hyporheic flows may interact with contaminants to affect stream biota. This research investigates the effects of hyporheic upwelling on the amphipod, *Hyalella azteca,* when exposed to zinc contaminated sediments. Elevated zinc concentrations are often observed in urban stream sediments as a produce of stormwater runoff and legacy metal contamination. In this experiment, we manipulated hyporheic upwelling and zinc contamination in flow-through artificial streams over time. Artificial streams allowed for greater control and manipulation of hyporheic flows and test conditions, to tease apart effects of hyporheic upwelling. *Hyalella azteca* survival was lowest in streams with both zinc and hyporheic upwelling, compared to streams with zinc contamination only. The results highlight the importance of hyporheic flows in altering metal bioavailability to benthic macroinvertebrates, and suggest future assessments of hyporheic flows in in situ ecotoxicology research.

WP090 The use of Near Infra-red Spectroscopy for measuring energy storage components of protein, lipid and glycogen in common bivalve species in Australia

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The function and sustainability of organisms, populations and ecosystems are driven by the input, use and transfer of energy. The storage and transfer of energy in organisms is influenced by environmental stress from physiochemical changes and contamination. Cellular Energy Allocation (CEA) is a measure of energy stores (protein, lipids and glycogen) versus energy consumption and provides a measure of organism response to stress. The capacity to rapidly assess CEA would provide a valuable tool to assess organisms' responses to both physiochemical stressors such as temperature, salinity, pH and oxygen changes and anthropogenic influences. Near Infra-red spectrometry (NIRS) quantitative modelling provides an approach that can provide high accuracy results with rapid results outputs and minimal sample preparation. In Australian estuaries, bivalves are a valuable monitoring species due to their filter feeding and sessile nature as well as aquaculture values. This study will present an approach for a rapid energy determination method that assesses energy storage in five bivalve species. NIRS models have been developed to assess energy stores of protein, lipid and glycogen for five bivalve species (*Saccostrea glomerata* (Sydney Rock Oyster), *Ostrea angasi* (Flat Oyster), *Mytilus galloprovincialis* (Australian Blue Mussel), *Crassostra gigas* (Pacific Oyster and *Anadara trapezia* (Sydney Cockles).

Screening and Prioritization Methods for Characterizing Risk of Contaminants in the Environment

WP092 Survey of polymers used in cleaning products: Categorization, Data Availability, and Prioritization of Polymers for Ecological Risk Assessment

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Polymers are molecules containing a number of repeating (monomer) units bonded together. They are utilized in a wide variety of products found in the built human environment, including cleaning products. Polymers are often exempt from chemical regulatory programs, e.g., for polymers above certain molecular weights in the US; in Europe, all polymers are exempt under REACH regulations. Regardless, understanding environmental impact throughout each stage of a products' life cycle is essential for good product stewardship and there is increasing interest in greater transparency in consumer product safety. For this work, publically available data were mined for environmental safety information for polymers used in cleaning products. Firstly, a definition of 'polymer' was established by harmonizing across international governmental definitions and industry conventions to identify chemicals within scope. Polymers in current use in cleaning products and formulations were compiled into a database, and chemical characteristics were catalogued. A key parameter differentiating various polymers was solubility; however, the form of some polymers may change during product formulation. Soluble polymers in this database were classified based on subcategory of polymer chemistry or function. This database initially included 181 polymers, but was prioritized and refined to focus on materials currently in common use and for which publicly available safety information was less common. The final list contained thirty polymers in ten chemical classes. Following a detailed literature search for each polymer, data gaps and prioritization for ecological risk were assessed. The data for each polymer were categorized into: fate and transport data (sorption, biodegradation, bioaccumulation, removal), and acute and chronic invertebrate, fish, and algal toxicity data. Publicly available data were not identified for most of these polymers for use in ecological risk assessment. For prioritization, two of the ten chemical classes (poly-quaternary polymers and polycarboxylates) were recommended as high priority for further study. Six classes were moderate in priority (ethers, glycerides, polyethyleneimines, polyols, polyesters, and melamine resin). Two classes containing polybutene and butanedioate (starch) were assigned low priority. The available data as well as the classification scheme and proposed areas for further research will be presented.

WP093 Use of Read-across for Testing Estrogen, Androgen, Thyroid, and Steroidogenesis Pathways in Vertebrate Ecological Receptors

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Testing programs developed by the U.S. Environmental Protection Agency (USEPA), the Ministry of the Environment in Japan, and the Organisation for Economic Co-operation and Development (OECD) have been designed to evaluate whether a chemical has the potential to interact with the endocrine system. Because of the high degree of conservation in estrogen, androgen, thyroid, and steroidogenesis (EATS) pathways among vertebrates, there is potential to extrapolate data generated for one vertebrate taxonomic group to others (*i.e.*, biological read-across). We reviewed the existing standardized toxicity tests for EATS pathways to determine the extent of coverage and adequacy of these existing test guidelines for decision-making and mode of action determination. Furthermore, the endocrine sensitive endpoints used for risk assessment of vertebrate wildlife and the potential for read-across between taxa was considered for each pathway. The limitations of using biological read-across approaches, which are rapidly evolving in the fields of computational biology and toxicology, were additionally reviewed. There are multiple axes and interactions that may affect EATS pathways. Available data from existing testing protocols show a high degree of confidence in the conservation of the hypothalamus-pituitary-gonadal (HPG) axis between fish and mammals and the hypothalamus-pituitary-thyroid (HPT) axis between amphibians and mammals. Comparatively, there is less support for the conservation of the HPG axis between amphibians and mammals, the HPG axis between birds and mammals, the HPT axis between fish and mammals, and the HPT axis between birds and mammals. Our review also found that more information on sensitive pathways and endpoints is needed to support biological read-across approaches for testing EATS pathways in vertebrate ecological receptors. Some of this information can be incorporated into existing protocols, while in other cases, new tools and techniques are needed. However, the future of these techniques is promising.

WP094 Prior Knowledge-based Approach for Associating Contaminants with Biological Effects: A Case Study in the St. Croix River Basin, MN, WI, USA

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Evaluating the potential human health and ecological risks associated with exposures to complex chemical mixtures in the environment is one of the main challenges of chemical safety assessment and environmental protection. There is a need for the development of approaches to integrate chemical monitoring and biological effects data to evaluate risks associated with chemicals present in the environment. Here, we used prior knowledge about chemical-gene interactions to develop a knowledge assembly model (KAM) for detected chemicals at five locations near the North Branch and Chisago wastewater treatment plants (WWTP) in the St. Croix River Basin, MN and WI. Site-specific KAMs were developed to generate hypotheses about the potential biological impacts of the chemicals at each location. Additionally, empirical gene expression data were also mapped to the assembly models to evaluate the likelihood of a chemical contributing to the observed biological responses using richness and concordance statistics. The integration of the gene expression data with the site-specific KAMs allowed for the prioritization of potential chemical contributors at each location. Atrazine was identified as a potential contributor to the observed gene expression responses at a location upstream of the North Branch WTTP. Four chemicals were identified as contributors to the observed biological responses at the effluent and downstream of the North Branch WWTP, with carbamazepine being a significant contributor at both locations. Four chemicals were identified as the greatest contributors to the observed biological responses in fish exposed to the effluent at the Chisago WWTP. Five chemicals were identified as contributors to the observed biological responses in fish exposed downstream of the Chisago WWTP, with 17-estradiol and estrone being two of the significant chemicals. Knowledge assembly models have strong potential for associating chemical occurrence with potential biological effects and providing a foundation for hypothesis generation to guide research and/or monitoring efforts related to the effects of contaminants in the environment.

WP095 EcoToxChip: A Toxicogenomics Tool for Chemical Prioritization and Environmental Management

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Chemical contamination of our ecosystems is regarded as one of the planet's greatest threats. Regulatory agencies and businesses are tasked with managing these chemicals but face significant challenges due to the sheer number of chemicals for which toxicity data are required. It has become increasingly apparent that current risk assessment strategies that rely heavily on animal testing and are prohibitively time-consuming and expensive are not able to address these regulatory mandates. We have recently been awarded a Large-Scale Applied Research Program (LSARP) grant from Genome Canada to develop, test, validate, and commercialize quantitative PCR- based arrays ("EcoToxChips"; containing 384 prioritized genes) to address an urgent worldwide need for advanced toxicity testing tools that are accessible, affordable, consistent, and reliable. To facilitate their adoption into standard practices we will produce and leverage social science knowledge, as well as provide a user-friendly bioinformatics portal (EcoToxXplorer.ca) and an end-user vetted technical guidance document. The anticipated socioeconomic benefits associated with the adoption of our deliverables, namely more focused animal testing, improved regulatory decision-making, and cost-efficiencies. Here we will provide a broad overview of our project.

WP096 Risk Assessment of Regenerative Thermal Oxidiser Air Emissions for Gas Treatment Plant in Australia

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A Regenerative Thermal Oxidiser (RTO) in Victoria State, Australia, processes benzene from nearby gas fields to meet Environment Protection Policy design criterion for benzene at sensitive receptors. Exceedance of the design criterion during routine emissions sampling occurred during operations and during RTO bypass emissions. Emissions during these same times were modelled along with future operational conditions with stack modifications and bypass emission scenarios. The modelling identified that the design criterion for benzene was exceeded at identified sensitive receptors. Therefore, a health risk assessment was carried out to demonstrate whether there are potentially unacceptable human health risks associated with exposures to site emissions under site-specific conditions. The scenarios included chronic human health risks to off-site receptors within the area, an assessment of acute (1-hour and 24-hours) human health risks to residents and workers at adjacent facilities, and an evaluation of potential risks to livestock cattle and chemical transfer into dairy products. Modelling was conducted using an approved regulatory version of AERMOD to assess potential benzene exposure point concentrations (EPCs) at the receptors. The EPC for other VOCs associated with the emissions was estimated using the ratio of each VOC to benzene at emission source. Based on the modelling conducted and the health risk assessment, potential chronic and acute risks associated with air emissions from the RTO under normal and modified operations and bypass conditions are considered acceptable. For all residential receptors and scenarios, the calculated incremental carcinogenic risk for the chronic scenarios were less than $1x10^{-5}$, and all calculated incremental carcinogenic risk for the proposed stack extension were less than $1x10^{-6}$. For noncarcinogenic risks, for all residential receptors, the chronic HI is less than 1. Benzene is the only carcinogen and is also the main risk driver for non-carcinogenic risk by 1 to 3 orders of magnitude. There were no unacceptable risks associated with acute exposures to benzene or other VOCs at the site with the 24-hour HQ for benzene and the 1 hour cumulative HI for all VOCs < 1. The environmental assessment did not show any potential risks to either human exposure to milk from dairy cattle or any adverse health effects directly to the exposed cattle.

WP097 Integrating Chemical Monitoring Data with Bio-effects Data to Prioritize and Predict Hazards of Contaminants of Emerging Concern in Minnesota Streams

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To identify and prioritize contaminants of emerging concern (CECs) in Minnesota's streams and rivers, surface water samples from 50 randomly selected locations across Minnesota were analyzed for pharmaceuticals, personal care products (PPCPs) and variety of other chemicals (a total of 146 chemicals). For a subset of 10 locations, exposure (site chemistry) and bioactivity datasets (Interactive Chemical Safety for Sustainability Dashboard) were integrated to predict biological targets of detected chemicals for each site. Lastly, 48h-exposure of adult fathead minnows to surface waters from 10 sites (n=7 fish per location) was conducted to determine whether predicted biological targets are induced in vivo; liver gene expression was analyzed using a custom 60K feature fathead minnow microarray. Fifty-eight of the 146 chemicals analyzed were detected at least once. Among the pharmaceuticals detected, iopamidol, an X-ray contrast agent, was the most frequently detected, found at 78% of the locations sampled. The antidepressants sertraline, amitriptyline, and fluoxetine were detected in water from 48%, 44%, and 10% of the locations, respectively, and the antibiotics sulfamethoxazole and erythromycin were detected in water at 24% and 14% of the locations, respectively. Metformin, a medication used to treat type II diabetes; triamterene, a diuretic; and carbamazepine, an anticonvulsive medicine, were detected at 18%, 16%, and 14% of the locations, respectively. The insect repellent DEET, the plastic component bisphenol A, the corrosion inhibitor benzotriazole, and benzotriazole breakdown products were also widespread. Dashboard-based predictions indicated that four streams contained concentrations of contaminants sufficient to initiate activation of variety of molecular targets; estrogen receptor alpha (ER) and peroxisome proliferator-activated receptors (PPAR; alpha and gamma) were activated at most stream sites. The predicted chemical initiators of these targets included bisphenol A, caffeine, carbamazepine, and triclosan. Exposure of fish to stream water indicated activation of 28 toxicity pathways (including above predicted PPAR and ER activation). Potential adverse outcomes of the activated pathways include impacts on reproduction, development, growth and tumor formation. Estrogenic effects remain of special concern as all methodologies indicated disruption or estrogen receptor binding and signaling.

WP098 Prioritization of organic contaminants in coastal environments using target and non-target screening analysis

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To date, more than 130 million chemicals have been registered in the Chemical Abstracts Service database. The widespread use of organic chemicals related with human activities has resulted in various adverse health effects to marine organisms. However, only limited chemicals are monitored or regulated by international and domestic authorities due to lack information on the existence and toxic effects of emerging organic contaminants in marine environment. To effectively manage emerging pollutants in marine environment, the prioritization procedure for emerging pollutants should be scientifically developed. In this study, we proposed the occurrence-based screening method for prioritization on emerging contaminants in marine environment, using non-target screening analysis (NTSA) with gas chromatography/time-of-flight. Seawater, sediment, and biota samples were collected from Ulsan Bay. Before the performance of non-target analysis, we established in-house database with standard solutions for 208 organic contaminants. The total ion chromatogram was used to identify target compounds matched with retention time and qualification ion based on the in-house library. After target analysis, the non-target analysis was performed to identify unknown chemicals in multi-media matrices. Mass spectrum of each separated peak from the deconvoluted ion chromatogram was compared with the spectrum information from NIST library. From the target analysis, the predominantly identified compounds were PCBs, OCPs, PCDD/Fs, PBDEs, PAHs, which have been monitored or regulated organic contaminants in Korean coastal environment. From the non-target analysis, several chemical groups such as siloxanes, phthalates, synthetic musks, and OPFRs were identified from multi-media samples, implying potential candidates as concerns of emerging contaminants for coastal environment of Korea. For weight of evidences, the NTSA was applied for 15 marine species with different trophic levels. With increasing trophic levels, several contaminants such as PAHs, PCBs, siloxanes, and OPFRs were biomagnified through marine food-web. These contaminants could be suggested as strong candidates for concerns of emerging contaminants in marine environment of Korea. Our approach or systematic framework proposed in the present study can be effectively utilized as a scientific-based decision procedure for prioritization on concerns of emerging pollutants in marine environment.

WP099 Characterization of Metabolic and Gene Expression Profiles in the Chicken Hepatocellular Carcinoma Cell Line, LMH, for Chemical Screening

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Due to the large back-log of chemicals that require regulatory evaluation, there has been a significant demand for high-throughput in vitro methods that rapidly generate mechanistic data for chemical and environmental risk assessment. Previous studies in our lab have utilized a primary chicken embryo hepatocyte (CEH) cell culture approach combined with biochemical and toxicogenomics assays (e.g. ethoxyresorufin-*o*-deethylase [EROD], PCR arrays) to screen and prioritize chemicals for follow-up whole animal toxicity testing; however preparing primary cultures is time-consuming and still requires the use of live animals. The aim of this study is to determine if the immortalized chicken hepatocellular carcinoma cell line, LMH, represents a suitable alternative to the CEH assay for chemical screening and prioritization. We compared the metabolic competence and gene expression profiles of LMH and CEH exposed to two dioxin-like compounds: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 3,3',4,4',5-pentachlorobiphenyl (PCB126). LMH exposures were conducted under three distinct culture conditions: 1) in proliferating cells; 2) cells grown to confluency and; 3) as three-dimensional spheroids in order to further characterize the optimal exposure conditions for chemical screening. EROD activity was used as a measure of metabolic competence and changes in mRNA expression were measured using a custom-designed chicken PCR array containing 27 TCDD-responsive genes. Compared to CEH, the use of the LMH cell line may improve the efficiency of generating mechanistic toxicity data while completely eliminating the reliance upon live animals for our in vitro toxicogenomic screening and prioritization program.

WP100 In Vitro Genotoxicity Screening in an Avian Hepatocellular Carcinoma Cell Line using Transcriptomics and Flow Cytometry

J. O'Brien, Environment and Climate Change Canada / National Wildlife Research Centre; S.P. Jones, Environment and Climate Change Canada / Books Acquisition; T. Sharin, D. Crump, Environment and Climate Change Canada / National Wildlife Research Centre

We regularly use toxicogenomics in primary avian cell cultures to screen and prioritize environmental pollutants of potential concern to wild bird species. However, primary cells are limited in their ability to identify agents that can cause genomic damage. This is largely because primary cells undergo very little DNA replication and cell division, which are two cellular functions generally required for most genotoxic modes of action. In contrast, continuous cell lines maintain an active cell cycle making them more sensitive to genotoxicity. In the present study, we incorporated an immortalized chicken hepatocellular carcinoma cell line, LMH, into our in vitro toxicogenomics screening pipeline. LMH cells were exposed to a variety of known genotoxic agents. Gene expression profiles were determined using chicken ToxChip polymerase chain reaction (PCR) arrays which contain probes for genes in a variety of toxicologically relevant pathways, including cell cycle regulation and DNA repair. Genotoxicity was also assessed using an in vitro flow cytometry assay that measures endpoints associated with DNA damage response pathways: cleaved PARP, γH2AX, and phospho-histone H3. In future studies we will test this system for its ability to detect the genotoxicity of chemicals that require metabolic activation. Successful incorporation of the LMH cell line into our in vitro toxicogenomics screening pipeline will provide the additional ability to detect genotoxic modes of action and significantly reduce the number of animals required for our chemical screening program.

WP101 Bioenergetics-Adverse Outcome Pathway (AOP): Incorporating energetic endpoints into risk assessment for chronic exposure scenarios

C. Goodchild, S. DuRant, Oklahoma State University

The adverse outcome pathway (AOP) framework is a helpful tool in ecological risk assessment. AOPs link toxicity across multiple levels of biological organization, and thereby facilitate the development of predictive biomarkers and provide the mechanistic information necessary for science-based extrapolation. Thus far AOPs have predominantly characterized acute toxicity; however, the AOP framework has not been well developed for characterizing effects from chronic exposure to contaminants. Although chronic exposure scenarios can alter an organism's energy budget, energetic endpoints are rarely incorporated into risk assessment because there is not an integrative framework for linking sub-organismal energetic effects to organismal and population-level responses relevant to risk assessment. In the present analysis, we developed a generalized bioenergetics-AOP in an effort to provide a framework by which energetic endpoints can be used to better inform risk assessment, specifically contamination scenarios that generate an energetic burden to animals. To do so, we conducted a literature review of energetic endpoints (e.g., cellular energetics and cellular energy allocation, metabolic rate, scope for growth), and ecological models relating individual energy-use to population-level effects (e.g., dynamic energy budget models). We argue that the bioenergetics-AOP framework facilitates the incorporation of energetic endpoints into risk assessment for chronic exposure scenarios and reduces uncertainty.

WP102 Health Benefit Values as Indices of Mercury, Cadmium, and Soft Electrophilic Agents

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Safety evaluations regarding methylmercury (MeHg), cadmium, (Cd), and other soft electrophile species (including multiple organic neurotoxic agents) are currently based on individual mass concentrations rather than applying their biochemically active molar concentrations. Although toxicant exposures are individually assessed, their complementary and/or synergistic effects on their biochemical target molecules need to be comprehensively considered to provide a reliably accurate indication of exposure risks. For example, studies of sentinel populations known to consistently consume meats of highly predatory sharks or whales have reported finding subtle dose-dependent effects on children exposed *in-utero*. However, studies involving consumption of typical varieties of ocean fish do not support the premise that maternal MeHg exposures result in adverse child outcomes. Instead, ocean fish consumption is directly associated with improved neurodevelopment. Exposures to MeHg has been found to be proportional to risk only in cases where MeHg concentration occurs in molar excess of selenium (Se) in the seafood consumed i.e., meats of highly predatory sharks or whales. Since these seafoods have very high MeHg, Cd, and organic toxicant burdens, and these soft electrophiles irreversibly inhibit the Se-dependent enzymes (selenoenzymes) required for normal brain functions, these studies indicate that the toxic effects of high MeHg and Cd exposures can be prevented by increasing dietary Se intakes, particularly through consuming Se-rich ocean fish. The Se-Health Benefit Value (HBV) criterion is a predictive indicator of dose-effect relationships between MeHg and Se concentrations in the fish or seafood. Negative HBV's predict relative risks (e.g., pilot whale; -83) while consumption of increasing amounts of ocean fish with positive values (e.g., yellowfin tuna; 15.7 ±3.4) predict benefits. Therefore, the HBV is a food safety indicator that reflects the beneficial nutrients provided by fish while allowing consideration of MeHg risks when present. The combined effects of MeHg, Cd, and organic toxicants with soft electrophile active groups on selenoenzyme activities need to be considered in concert. Epidemiological studies are likely to obtain more consistently reliable results if they consider combined effects of soft electrophile exposures in relation to Se status when predicting health outcomes.

WP103 Minnesota's Aquatic Toxicity Profiles

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The state of Minnesota has been collecting occurrence data for a highly diverse set of contaminants that includes pharmaceuticals and personal care products, flame retardants, and industrial chemicals for several years. Many of these contaminants have been detected at low concentrations in the aquatic environment across Minnesota. Toxicity data and associated regulatory or screening values for many, if not most, of these contaminants are lacking, which makes it difficult to put the occurrence data into context and characterize the potential impact of these contaminants to aquatic life. The Minnesota Pollution Control Agency wanted a way to characterize the available contaminant monitoring data in order to make informed decisions about its water quality, pollution prevention, and monitoring programs. The Aquatic Toxicity Profile (ATP) is a tool that uses a weight-of-evidence approach to gain a broad understanding of the potential impacts associated with the presence of specific contaminants in the aquatic environment. The ATPs are intended to be a non-regulatory, rapid screening approach to evaluate contaminants, utilizing both measured and modeled data about the chemical properties, occurrence, toxicity and potential mode of action.

High-Technology Metals as Emerging Contaminants of Potential Concern – Environmental Fate, Transport and Toxicity

WP104 Determination of speciation and solubility of Samarium and Dysprosium in Aquatic Environments

S. Brennan, Wilfrid Laurier University; D. Smith, Wilfrid Laurier University / Chemistry

The rare earth elements (REEs) are extremely useful due to their unique magnetic, catalytic and luminescent properties. However, their increased use poses concern with the poor understanding of their speciation and toxicity. Previous research has stated that the pK_{sp} values for REEs Samarium (Sm) and Dysprosium (Dy) are 25.5±0.9 and 25.9±0.9, respectively. With the associated errors spanning two orders of magnitude, the objective of this research is to use solubility tests to determine K_{sp} values with a higher degree of accuracy. These solubility constants were in turn used to model the speciation of these metals. Solubility tests were conducted at fixed pH values of 6, 7, 8 and 10 where 0.45mm filtered and unfiltered sub-samples were taken hourly for 8 hour periods. This time period was selected as it is consistent with toxicity test water renewal times. The total and dissolved concentrations were then measured using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). This analysis was useful in identifying signs of precipitation, as well as assessing the presence of toxic soluble species vs less harmful particulate matter. The total metal concentrations used were 1, 10, 25, 50, 75 and 100 mM. At a pH of 6, the total and dissolved concentrations differed by only 0.5-1% for all total metal concentrations measured, indicating that minimal precipitation occurred. This is in agreement with chemical equilibrium modelling software, which predicted that significant precipitation occurs at pH values higher than 8. The completion of these solubility tests and ICP-OES measurements will provide further insight into Sm and Dy precipitation equilibria and speciation, and lay the groundwork for the evaluation of their toxicity.

WP105 Impact of chromium on aquatic invertebrates and influence of water chemistry on toxicity responses

S. Choi, L. Mindorff, J. McGeer, Wilfrid Laurier University / Biology

The goals of this study were to evaluate the toxicity of chromium to the invertebrate *Hyalella azteca* and to characterize the potential toxicity mitigating influences of Ca, Na, Mg and dissolved organic matter (DOM). Acute 96 h exposures following standard methods (Environment Canada EPS 1 RM 33) were used. The responses of two groups of *H.azteca* were compared, one from a southern Canadian source (CCIW) and one collected from the Ring of Fire in northern Ontario. The Ring of Fire is an area with a large deposit of minerals, as yet unexploited but due for development in upcoming decades. *H.azteca* from both sources were cultured in a reconstituted medium in moderately hard water at 60 mg/L $CaCO_3$ and pH of 7.3. In this medium, the LC50 value was 120 µg/L (95 % confidence interval 155.22 to 88.56). It was hypothesized that DOM complexation would reduce toxicity, but that cations (Ca, Mg, Na) would not compete for uptake (and toxicity) of Cr. Initial tests showed that DOM (sourced from Pickle Lake, ON) at a concentration of 5mg DOC/L, did not reduce toxicity (LC50 of 128 µg/L with 95% CI 171.97 to 89.27). This study will contribute to an improved understanding of site specific chromium toxicity in the context of northern Canadian environments. Research is supported by Environment and Climate Change Canada and the NSERC Strategic Grants Program with contributions from the Ontario Ministry of Environment and Climate Change and Avalon Rare Metals, Inc.

WP106 Toxicity of Novel 2D Manganese Oxide Nanosheets in Fish Cells

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The development and production of engineered metal oxide nanomaterials continues to increase exponentially, increasing the risk of their release into the aquatic environment. The practice of "safety by design" aims to reduce the toxicological impact of engineered nanomaterials by assessing their toxicity during development. Here, we employ this approach utilizing a model fish gill cell line to represent a target tissue of freshwater fish likely to be exposed to nanomaterials in the environment. Using this cell line, we are determining the toxicity of novel 2D manganese oxide (MnO_2) nanosheets currently under development for use as catalysts and in supercapacitors or batteries. Our preliminary data show MnO_2 nanosheets undergo reductive dissolution in the presence of biological reducing agents such as glutathione. Thus, we compare the toxicity of the MnO_2 nanosheets to that of soluble $MnCl_2$ and insoluble MnO_2 particles in order to assess the impact of nanoparticle solubility on toxicity. Preliminary results suggest the 2D MnO_2 nanosheets are more cytotoxic than either MnO_2 particles and soluble $MnCl_2$. Since manganese is known to induce toxicity by dysregulating mitochondria, we are investigating how the uptake and dissolution of these 2D nanosheets impacts mitochondrial shape and function. Our preliminary results suggest mitochondrial activity is inhibited by manganese nanosheets at sub-cytotoxic concentrations. Together, this study will allow us to predict the environmental toxicity of this novel nanomaterial while it is still under development. This research is supported by NIEHS Superfund Research Program P42 ES013660 and NIH F32 Training Grant ES00727225.

Stormwater and Wastewater Pollution – Toxics, Ecological Sensitivity and Sustainable Solutions for a Healthy Environment

WP107 Toxicity of Roofing Runoff and its Effects on Coho salmon and other aquatic organisms

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The impact of stormwater runoff to aquatic ecosystems and the use of Green Stormwater Infrastructure (GSI) to remediate this impact are increasing important areas of scientific study particularly in areas of the world affected by heavy rain during the winter months. Salmon are commercially and culturally important species to the Pacific Northwest United States. Many wild salmon populations are in decline with specific populations categorized as threatened or endangered. There have been reports in recent years of large die-offs of Coho salmon in small rivers and streams; these deaths often occur before spawning furthering the decline of the population. Urban runoff has high levels of various pollutants that have been shown to cause negative effects on several aquatic species, including salmon. A variety of roofing materials contain metals, as well as organic and inorganic chemicals. These chemicals have been shown to leach during rain events, ultimately ending up in stormwater, which then enters rivers and streams and eventually salt water systems. In this study, we investigated the capacity of several roofing materials to leach pollutants during rain events, in the Puget Sound region of Washington State. We also evaluated the toxicity of runoff from these roofing materials to three aquatic species, the water flea, Cerodaphnia dubia, Zebrafish embryos (*Danio rerio*), and Coho salmon, (*Oncorhynchus kisutch*). The results of this study will provide insight into the potential effects of roofing materials on salmon and some of their prey items as well as the chemical loading of roofing materials into bodies of water over an extended time period.

WP108 Physiologic indicators after short-term exposure to stormwater runoff in adult coho salmon vulnerable to lethal effects

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Coho salmon (*Oncorhynchus kisutch*) return from the Pacific Ocean during autumn to spawn in lowland streams of the Pacific Northwest of North America, including Washington State. In urban areas bordering the Puget Sound in Washington State, high rates of pre-spawning mortality (PSM) have been documented in coho salmon spawners. While the precise cause of PSM is still unknown, PSM has been correlated with roads and density of vehicle traffic in the watershed – supporting the hypothesis that PSM is caused by stormwater running into salmon bearing streams from impervious surfaces, particularly heavily used roads. Coho PSM can be experimentally induced by exposure to urban road stormwater runoff, demonstrating a progressive suite of symptoms synchronous with those observed in the field that include lethargy, disorientation, loss of equilibrium, and death within 4 hours of exposure. The present study exposed 68 coho salmon to runoff from an urban arterial roadway and 43 to clean well water across 6-storms in the fall of 2016. Behavior and hematology (arterial and venous blood samples analyzed for ions, blood gases, and metabolic markers) was evaluated after 30 minutes of exposure (termed: pre-symptomatic) to gain insight into the pathophysiology underlying early stages of coho urban pre-spawn mortality. Fish were also sacrificed and sampled after a loss of equilibrium was observed (termed: symptomatic). Preliminary data demonstrates a significant imbalance of ions (sodium, potassium, and calcium) in the runoff exposed fish compared to the control fish after 30-minutes of exposure. No other measured blood metrics were different between the two groups. The symptomatic fish exposed to runoff, however, demonstrated ion imbalance and modulated blood gases and metabolic markers. The observed ion imbalance was apparent in the arterial blood, but not the venous blood in both the pre-symptomatic and symptomatic fish indicating the gill may be involved in this process. At this time, neither the toxic chemical nor underlying cause of death involved in PSM is known. This study evaluated pre-symptomatic physiologic indicators in adult coho salmon exposed to stormwater from highway runoff,as a first step to elucidate which physiologic indicators are initially modulated during the progression of symptoms from this lethal exposure.

WP109 Toxicity of a major wastewater discharge to freshwater mussels

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In November 2015, controlled release of large amounts of untreated wastewaters was done in the Saint-Lawrence to permit critical upgrades in the sewage system. This opportunity enabled us to study the impacts of run-off water and untreated wastewaters in the context that sewage overflows situation by increased precipitation events (climate changes). Water samples were collected at 10-25 m of 6 discharge sites for exposure to quagga mussels for 96h at 15^{o}C. Mussels were also exposed to downstream river site (30 km from the first emission site) and aquarium water as controls. After the exposure period, mussels were processed for xenobiotic biotransformation (CYP1A and glutathione S-transferase activities), estrogenic effects (vitellogenin-like proteins), mitochondria activity /triglycerids, genotoxicity (DNA strand breaks) and lipid peroxidation (LPO). The data revealed that exposure to the waters increased CYP1A1 activity and vitellogenin-like proteins. Mitochondria activity (electron transport) and triglycerids were significantly reduced with no evidence of LPO. DNA strands breaks were also significantly decreased suggesting decreased DNA turnover. Multivariate analysis revealed that CYP1A activity, DNA strands and tryglycerids were the most important endpoints explaining 70% of the total variance where the controls and downstream river water were readily separated with emission sources. In conclusion, mussels exposed to untreated wastewaters and runoff waters had increased CYP1A activity with decreased DNA turnover and tryglycerids where only DNA effects were detected at the farthest downstream sites.

WP110 Overview of research on acute aquatic toxicity from urban stormwater runoff: Collaborations of the Puget Sound Stormwater Science Team

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The Puget Sound Stormwater Science Team is a collaborative research effort by NOAA-NMFS, USFWS, and Washington State University to understand and prevent toxic impacts from urban stormwater runoff. Urban stormwater runoff is acutely toxic to a variety of aquatic animals, including model species such as the waterflea *Ceriodaphnia dubia*, and the embryo-larval zebrafish (*Danio rerio*), as well as non-model species. There are five species of the genus *Oncorhynchus* native to the Pacific Northwest of the USA. Coho salmon (*O. kisutch*) is particularly sensitive to urban stormwater runoff. Anadromous adults returning from the ocean to spawn in freshwater show high rates of pre-spawning mortality in streams that receive runoff from impervious urban surfaces – particularly roads. Experimentally, runoff from roads is sufficient to cause acute mortality in coho adults, juveniles, and hatched embryos. Prior to mortality, a variety of impairments are observed in affected individuals, including an innate immune response, ionoregulatory impairment, and hemoconcentration. Ongoing work strives to understand the cause of death in exposed coho, the extent of impact in regional streams, toxic responses in other members of the aquatic ecosystem, the source and chemical identity of the causative agent(s), and green infrastructure approaches that can mitigate the toxic responses.

WP111 Multi-component analysis to detect stress-induced hemoglobin derivatives in coho salmon (Oncorhynchus kisutch)

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Coho salmon (*Oncorhynchus kisutch*) exhibit pre-spawn mortality and cardio-respiratory distress linked to stormwater runoff in Puget Sound. A wide variety of toxicants potentially present in stormwater can elevate levels of fish methemoglobin, the oxidized form of hemoglobin that impairs oxygen transport. Methemoglobin may also serve as an indicator of other possible lethal and sub-lethal effects of oxidative stress. Current methodologies for methemoglobin determination—multi-wavelength and cyanide addition methods— give rise to mathematical errors and spectral interferences when applied to coho salmon. To overcome these challenges, we experimentally determined molar absorptivities for the coho hemoglobin derivatives oxyhemoglobin, deoxyhemoglobin, carboxyhemoglobin and methemoglobin, and used them in an adapted spectrophotometric multi-component analysis. Our results indicate that while oxy, deoxy and carboxyhemoglobin molar absorptivities appear similar in humans and coho, methemoglobin molar absorptivities are significantly different ($P = 0.0019$ at $\lambda = 630$ nm). Standard multi-wavelength methods, such as co-oximetry, when applied to coho salmon can give rise to an artificially low estimation of methemoglobin and over-estimation of carboxyhemoglobin on the basis of mathematical errors when human molar absorptivities are assumed. These results demonstrate a multiple linear regression least-squares fit incorporating the entire spectral range (450–700 nm) using molar absorptivities determined for coho is an ideal method to determine the concentration of methemoglobin in coho salmon and can be adapted for other fish species.

WP112 All's Swale That Ends Swale: Treatment of Toxicants in Pacific Northwest Highway Runoff Using Compost Amended Biofiltration Swales

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Biofiltration swales, or bioswales, use vegetated soil substrates to filter contaminants from stormwater, decrease sediment load, and reduce erosion. Following a storm, runoff moves slowly through the swale at a shallow depth. While stormwater is retained in the bioswales, pollutants are removed by the combined effects of filtration, infiltration, settling, and biotransformation. The system currently being evaluated at WSU Puyallup Research and Extension Center uses compost to further enhance the ability of bioswales to remove toxicants. Washington State Department of Transportation (WSDOT) has created guidelines for constructing compost amended biofiltration swales (CABS) in the Highway Runoff Manual for Stormwater Best Management Practices (BMP). In 2009 WSDOT implemented a field test for CABS along Washington State Route (SR) 518, north of SeaTac International Airport. Stormwater treatment efficacy of CABS compared to traditional bioswales were evaluated by Herrera 2011 and found to be more effective at removing dissolved metals. As part of an ongoing study, influent and effluent samples are currently being collected at the field site during storm events and tested for metals, PAHs, pesticides, phthalates, unknowns (LC-QTOF), and toxicity (zebrafish and daphnia bioassays). A laboratory model for CABS was developed at WSU Puyallup REC to verify field results in a controlled setting and identify ways that the WSDOT design can be adapted to address the wide varieties of issues presented by stormwater pollution. The laboratory model is representative of three ~50ft CABS longitudinal cross-sections that are 4-inches wide. Native top-soil was used as the substrate for a 3-inch blanket of compost placed within the bioswales. The CABS were then inoculated with a grass seed mixture consisting of red fescue (*Festuca rubra ssp. rubra*), meadow foxtail (*Alopecurus pratensis*), and white dutch clover (*Trifolium repens*). Slope and stormwater flow rate for these CABS was adjusted to test a variety of field conditions and retention time dye tests were used to calibrate the system to WSDOT specifications and understand the hydrological characteristic of stormwater in CABS. Finally, stormwater from a roadway with high traffic volume in Seattle, WA (SR-520) was applied to the laboratory CABS and samples were analyzed for chemistry and toxicity. Results from this study show how field and laboratory tests can be used to determine the effectiveness of CABS as a BMP.

WP113 Bioeffects-Based Approach to Evaluate Attenuation of Stormwater Toxicity by Best Management Practices

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This study aims to characterize the toxicity of urban stormwater and determine if specific best management practice applications (i.e., iron-amended sand filters) can attenuate stormwater toxicity. To achieve this, we utilized a battery of in vitro, high-throughput cell-based assays that can measure cumulative toxicity and indicate potential mechanisms of toxicity (particularly, chemical-molecular interactions that initiate toxic responses) in water samples. Stormwater samples were collected on four occasions (during major snowmelt and rain events) from three stormwater outfalls, and from the three sand filter inlets and their respective outlets in the metropolitan area of Minneapolis and St. Paul, MN. We evaluated the effects of stormwater samples on 25 human nuclear receptors and 46 transcription factors with Attagene FACTORIAL™ technology. Additionally, the unmodified, DeltaTox® II, low-toxicity B-Tox protocol was used to evaluate acute toxicity of whole stormwater. Stormwater samples generally had very low acute toxicity (median inhibition of 5%) and none of the inhibition demonstrated by sand filter inlets was present in corresponding outlets. High-throughput in vitro assays indicated that stormwater typically upregulated approximately a dozen of molecular targets including: the peroxisome proliferator-activated receptors (PPARs) gamma and alpha, the estrogen receptor alpha, the pregnane X receptor, the arylhydrocarbon receptor, and the Nrf2-antioxidant response element signaling pathway. The magnitude of upregulation in the majority of the stormwater samples (untreated and sand filter treated) was similar to or greater than that of treated municipal wastewater effluent samples from a major metropolitan area. Adverse impacts on reproductive development, endocrine and liver function may result from this upregulation. In most cases, passage of stormwater through sand filters reduced toxicity (e.g., arylhydrocarbon receptor, PPAR gamma and alpha), but in several cases the toxicity was unchanged (e.g., pregnane X receptor, estrogen receptor alpha). We are currently integrating in vitro, analytical chemistry, and in vivo *Daphnia* results to identify chemical drivers of stormwater toxicity and determine whether observed reductions in the toxicity "carry over" to the in vivo scenario.

WP114 Assessment of the impact of plants and fungi on bioretention performance for stormwater management

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New permits in Washington State for the National Pollutant Discharge and Elimination System (NPDES) are requiring the use of low impact development (LID) and green stormwater infrastructure (GSI) where feasible. Bioretention—infiltration into soil—is expected to be among the most commonly utilized techniques. Previous replicated mesocosm studies at Washington State University (WSU) and the Washington Stormwater Center (WSC) indicate that while bioretention soil can dramatically reduce the toxicity of urban stormwater, the role of plants appears limited to aesthetics and hydrologic performance. Recent studies have also indicated that wood-decomposing fungi can be incorporated into the wood mulch used in bioretention and can provide unique environmental services such as enhanced removal of microbial pathogens and degradation of PAHs. A multi-year WSU/WSC study is underway to evaluate the effects of the deciduous shrub Pacific Ninebark (Physocarpus capitatus) and the mycelium of the white rot saprobic Wine Cap mushroom (Stropharia rugoso-annulata) on the toxicity and water quality of bioretention-treated effluent under field conditions. Four treatment mesocosms (no plants / no fungi; plants / no fungi; no plants / fungi; plants / fungi) have been installed in triplicate in Seattle, WA and receive runoff in real-time from a busy interstate highway as part of an urbanized watershed. Quarterly sampling of influent stormwater and treated effluent over two years is in process to determine the potential for toxicant break-through under field-relevant loading conditions. Known stormwater toxicants including PAHs, Cu, Zn, and Pb, among others, are being monitored and toxic effects in zebrafish (Danio rerio) embryos including survival, developmental abnormalities, and cardiac function after 48 h exposure are being assessed. Results from the first 9 months of the study are reported.

WP115 Bioavailable phosphorus in pulp and paper mill effluents: Findings from algal growth assays

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A key aspect of receiving water quality assessment (i.e. TMDL, WLA) is linking the pollutant source to the environmental target of concern. When assessing phosphorus (P) as a pollutant, environmental targets are often associated with excessive algal growth, and establishing a quantitative phosphorous-algae link allows the calculation of necessary phosphorus source reductions that restore water quality to appropriate levels. Complicating this calculation is that the forms of phosphorus present in non-point source runoff, wastewater effluents, and the receiving water itself may not be directly available for algal growth, or they may become available only after a long period of time in the receiving water. We conducted a study to quantify and characterize the bioavailability of phosphorus in pulp and paper mill effluents using an algal assay in which vessels containing effluent or control solutions and non-P nutrients were inoculated with P-starved algae and placed under ideal light and temperature conditions until growth ceased. Algae were removed from test solutions, and the vessels reinoculated with P-starved algae and placed under ideal growth conditions. This repeated addition and removal of algae continued until algae ceased to grow, with algae removing bioavailable P, and the non-bioavailable P determined by the residual phosphorus remaining at the point algal growth no longer occurs. Bioavailable phosphorus is determined as the difference between the sample's initial phosphorus concentration and this non-available phosphorus. Assays conducted with four separate effluents demonstrated that uptake of available P can take as long as 36 days, and 4-6 cycles of adding and removing P-starved algae for growth to cease. Nutrient analysis is still underway for two of the four effluents, but findings from two effluents show that 15-20% of effluent P present was unavailable for algal growth. Ultrafiltration of final test solutions suggests that a significant amount (80-90%) of non-bioavailable P may be colloidal in nature and possibly associated with non-available or slowly available humic metal-phosphorus complexes. These findings have important implications for the development of more effective nutrient-response linkages, and successful water quality management targets.

WP116 Effects of municipal wastewater effluent on fathead minnow hepatic proteome

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Urban wastewater and runoff are a major source of contaminants in aquatic environments. Possible detrimental effects of exposure to contaminants include compromised performance to secondary acute stressors. Chronic stress, including exposure to contaminants, modulates corticosteroid levels, and cortisol is the primary corticosteroid in teleosts. One of the primary sites of cortisol action is the liver, where it regulates a variety of metabolic pathways in order to reestablish homeostasis in response to acute stressors. In order to investigate the impact of municipal wastewater effluent (MWWE) within and downstream of urban environments, the liver proteome of fathead minnows (Pimephales promelas) caged at various sites in the Bow River, Calgary, Alberta, was analyzed. Liver samples were collected from fathead minnows caged in five sites along the river, two upstream and three downstream from wastewater treatment plants. Proteins isolated from the samples were labeled using isobaric mass tagging and mass spectrometry was used identify and quantify proteins in the samples. Differences in protein abundance between samples were compared to determine the impact of MWWE on liver physiology between the sites, particularly in pathways related to stress response, metabolism, and energy partitioning. Acknowledgement: This study was supported by the Natural Sciences and Engineering Research Council of Canada Strategic Project Grant to MMV and BJM.

WP117 Analysis of the impact of Estrogen on abundances of fathead minnow and bluegill sunfish in Minnesota

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Wastewater treatment plants discharge effluent containing estrogens. These contaminants have caused dramatic ecological effects such as fish feminization and fish population collapses, with unknown long-term consequences. Estrone, an estrogenic hormone naturally secreted by women, is the most important estrogen that flows out wastewater treatment plants. Although this and other estrogens are present in Minnesota lakes and rivers and can be ecologically harmful, their treatment and discharge are not regulated. Nevertheless, discharge of estrone and other estrogens is linked to nitrogen removal which is regulated to some extent in Minnesota. The nitrogen discharge will be more heavily regulated in the future, requiring additional wastewater treatment plant upgrade. This project is part of a research program which aims to (1) determine the performance of different wastewater treatment processes with respect to nitrogen removal, estrone removal, energy use, and cost; (2) determine how temperature and life stage alter the reproduction and survival of fathead minnow (*Pimephales promelas*) and bluegill sunfish (*Lepomis macrochirus*) after exposure to treated synthetic or real wastewater; (3) conduct a cost-benefit analysis that links the cost of different wastewater treatment options to mathematical predictions of fathead minnow and bluegill sunfish abundance. The main goal of this research program is to identify the very best wastewater treatment strategy for the protection of Minnesota's natural resources. In the present project, we used data at individual and sub-individual scales to calibrate a bio-energetic model for the fathead minnow and bluegill sunfish. This individual scale model is based on Dynamic Energy Budget (DEB) theory and integrates effects of temperature and estrogen exposure. We implemented this model in an Individual-Based Model (IBM) that uses environmental cues (e.g., seasonal temperature) of a pristine Minnesota River. This methodology takes into account the effects on individual energetics as well as effects on behavior and allowed us to infer the impacts of estrogen exposure on fish at the population scale, which is a more relevant scale regarding ecological management and protection.

WP118 Evaluating the distribution and spatial persistence of estrogenic effects on fish below municipal wastewater treatment plants

J.R. Anderson, D. Winkelman, Colorado State University / Fish, Wildlife, and Conservation Biology; A. Jastrow, USEPA / Region 5

Complex chemical mixtures in effluents below municipal wastewater treatment plants (WWTPs) are difficult to assess and their effects on organisms are poorly understood. In the South Platte River basin in Colorado, estrogenic impacts from treated municipal wastewater have been observed, but the distribution of these impacts is poorly understood. Our goal was to gain a better understanding of the distribution and spatial persistence of estrogenic effects downstream of WWTPs in the South Platte River drainage. To understand the distribution of potential estrogenic effects, we caged male Fathead Minnows above and below effluent discharges at ten WWTPs in the upper S. Platte River drainage during the summer and fall of 2015. We placed ten male Fathead minnows in both upstream and downstream cages for one week, and then euthanized them, and extracted their livers for Vitellogenin (VTG) analyses. The presence of VTG is a common biomarker for estrogen exposure in male fish, and VTG presence was determined using qPCR. We observed VTG expression at two of the ten treatment plants. To determine the consistency of VTG expression, in 2016 we repeated the caging process used in 2015 at four of the WWTPs (two that showed VTG expression and two that did not). The results from the 2016 caging were very similar to the 2015 caging, with VTG expression still present where it was observed the first year and absent where it was not seen the first year. To evaluate the spatial persistence of downstream estrogenic effects, we caged fish at intervals downstream at the two WWTPs where we observed VTG expression. Downstream VTG persistence varied between the two WWTPs. At one, we observed VTG expression over 3200 m from the effluent outfall and at the other the VTG signal was absent within 400 m of the effluent. The spatial persistence of estrogenic effects is context dependent and is probably due to the level of dilution. In our case, one effluent is diluted as it enters a river and the other is nearly 100 percent effluent. However, we need further research to understand the mechanisms underlying the spatial persistence of estrogenicity. Our study indicates that estrogenic effects are spatially variable in the South Platte River drainage and the spatial persistence of these effects is dependent on ecological context. Our results provide a necessary template for further studies to understand the population and community consequences of estrogenic exposure.

WP120 Carcinogenic PAHs and Management Decisions about Urban Stormwater Pond Sediment in Minnesota

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In Minnesota, municipal stormwater pond sediments must be analyzed for a suite of 17 carcinogenic PAHs (cPAHs), 10 noncarcinogenic PAHs, arsenic, and copper prior to dredging. The cPAHs, using benzo[a]pyrene (B[a]P) equivalents, often drive upland management decisions about dredged sediment when compared to state Soil Reference Values (SRVs). The Minnesota Pollution Control Agency (MPCA) is the state agency responsible for regulating certain urban stormwater ponds and developing SRV values. While the MPCA recognizes that stormwater pond sediments contain a mixture of contaminants, other organic contaminants are thought to be of less concern than cPAHs. That is, if the B[a]P equivalents are high enough to require disposal of the dredged material in a lined landfill, then this will also take care of other unknown contaminants that may also be elevated in the sediments. This assumption was based on conventional contaminants (e.g., PCBs). However, little is known about the presence of emerging contaminants in stormwater ponds located in Minnesota. To this end, the MPCA collected surficial sediments from 15 stormwater ponds in the Minneapolis-St. Paul, MN metropolitan area during October 2009. Five ponds from each of the following major landscape categories were sampled: residential, commercial, and industrial land uses. Composite samples were analyzed for a suite of 18 metals and metalloids, mercury, cPAHs, 34 parent and alkylated PAHs, several emerging contaminants (i.e., alkylphenols and alkylphenol ethoxylates, PBDEs, per- and polyfluoroalkyl substances, and pyrethroids), SVOCs, chloride, total organic carbon, black carbon, and particle size. The PAH data from this study were published using an older method for calculating B[a]P equivalents. These data have been re-evaluated using the MPCA's updated method. Principal components analysis (PCA) will be used to determine if B[a]P equivalents account for most of the variability of the data for parameters detected in all of the samples. For the emerging contaminants, 4-nonylphenol and BDE-209 were the only analytes detected in all pond sediments, and median values were significantly higher ($p< 0.05$) in ponds from industrial areas compared to ponds from residential areas. Hierarchical cluster analysis of the PCA component scores will be used to determine if ponds with similar watershed land uses cluster together. An integrated assessment of the results will be provided with recommendations for future action.

WP121 Enhanced Removal of Phosphorus Using Anoxic Zones in Sewage Treatment Facilities: Performance Evaluation and Its Impact on Receiving Water Quality

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Increased urbanization and intense agricultural activities have overburden many urban ecosystems, resulting in eutrophication and algae bloom in streams and reservoirs. The endogenic phosphorous is of particular troublesome because it cannot be removed via gas from water-sediment by any chemical and biological mechanism. Our study site League City is located in the Greater Houston-Galveston area and is the 2nd fast growing city in the U.S. Surrounding streams and the Galveston Bay receive a significant portion of nutrients from domestic sewage treatment plants; therefore, total phosphorous (TP) removal is a primary concern for all wastewater treatment facilities within this urbanized area. Monitoring data (2014-2016) were collected from two facilities in order to evaluate the performance of TP removal using enhanced biological phosphorous removal (EBPR) through the engineered incorporation of anoxic zone. This field-scale project compared TP removal in two facilities with similar wastewater characteristics/environmental conditions but one difference (Plant A has an anoxic zone whereas Plant B does not). Results showed that anoxic zone significantly improved TP removal by 30%, suggesting its benefits to microbial uptake of nutrients. TP removal was significantly higher in Plant A, yielding an average 96% removal efficiency and effluent TP of 0.80 mg/L, which is below the acceptable limit regulated by Texas Pollutant Discharge Elimination System (TPDES) permit requirements. TP removal in Plant A was highly correlated with influent COD (Pearson's $r = 0.913$), suggesting high organic loading (influent COD) is essential to maintain the low DO in the anoxic zone. Plant A has been operated for ~ two years with stable performance and low maintenance cost. Plant A discharged effluent to Clear Creek Tidal Segment No. 1101 of the San Jacinto-Brazos Coastal Basin which is heavily used by recreational activities (fishing, boating, skiing, and swimming). Due to the small flow of receiving river, our mass balance calculation further reveals that effluent TP is almost equivalent to the TP in the receiving water (0.80 vs. 0.67 mg/L). This full-scale project demonstrated the success of anoxic zone in conventional activated sludge system. EBRP has its advantage of lower cost and less sludge; however, the cost-effectiveness of modifying existing activated sludge systems and improving receiving water quality as part of the TMDL measures need further justifications.

WP122 Determining the Presence of Traditional and Alternative Fecal Indicators in Murfreesboro, TN Storm Drain Biofilms

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Escherichia coli (*E.coli*) is a traditional fecal indicator bacteria (FIB) used by regulators to assign impairment to recreational waters. One drawback to use of *E. coli* as an FIB is its ability to multiply in the environment outside of the host gut. Evidence exists for *E. coli* proliferation in biofilms within storm water infrastructure. Dislodged fecal bacteria from biofilms transported to a waterway could prompt or maintain federal impairment status (303-d listing) without recent fecal inputs. Because of this, the USEPA has developed methods for detection of alternative indicators of fecal pathogen impairment with minimal replication outside of the gut, such as bacteria in the order *Bacteroidales* and viruses known as coliphages. The objective of this study is to determine the presence of the *E. coli*, F? and somatic coliphages, and *Bacteroidales* in biofilms in storm water drains near outfalls and compare their concentrations. Biofilm samples were collected once from outfalls for three impaired (303-d listed for *E. coli*) Murfreesboro, TN streams between 48 to 96 hours after different rain events (>0.15"). The concentration of *E. coli* in storm drain biofilms was measured using Colilert by IDEXX, coliphages using the single agar layer (SAL) procedure (EPA Method 1602), and human-specific *Bacteroidales* genetic markers were assessed by quantitative polymerase chain reaction (qPCR) at 48 and 96 hours. Relationships between *E. coli* and coliphage concentrations were investigated by Pearson correlation analysis. *Escherichia coli* was detected at high concentrations in the storm water outfalls (3.89 ± 1.13 log MPN/g biofilm (n=5)), with no observable correlation with F? coliphages (0.403 ± 0.388 log PFU/g biofilm; $R= 0.222$; $p=0.720$ (n=5)) or with somatic coliphages (0.729 ± 0.308 log PFU/g biofilm; $R= -0.203$; $p=0.743$ (n=5)). Concentrations of human-specific *Bacteroidales* were statistically higher at 48 (4.94 ± 0.29 log copies/g biofilm) than 96 hours (4.56 ± 0.066 log copies/g biofilm) post-rain event, implying a lack of proliferation. The lack of correlation between E. coli and coliphages in this study may imply that coliphages will not be useful indicators in biofilms or that the proliferation of E. coli in the storm water infrastructure is masking any correlations that initially existed.

WP123 Effects of a temporary raw urban wastewater discharge on environmental metal contamination and the biology of the freshwater crustacean Daphnia magna

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In November 2015, 4.9 billion liters of untreated wastewater were diverted from the Montreal's wastewater treatment plant and discharged into the St. Lawrence River, QC, Canada over the course of four days.

These discharges were done to enable the repairment of a 30-kilometre-long sewage interceptor. In order to evaluate the metal contamination and effects of these untreated effluents on the biology of invertebrates, surface water samples were collected at 4 specific points of discharge and 4 locations downstream of the river during the discharge event and a month after it ended. The levels of 27 trace metals and inorganic contaminants were measured in water samples. Results indicated that the concentrations of 22 of the elements measured were significantly higher during the event compared to several weeks after it ended. One site near a discharge point in particular, showed that the concentrations of Cu, Zn, Pb, Mo and Bi during the event were 2 to 37 times higher than at the other sites. Freshwater crustaceans *Daphnia magna* were exposed for 13 days to the surface water collected from three discharge and one downstream sites. Life-history endpoints (i.e., survival, reproduction, and growth) were measured and the transcription and activity of a suite of genes and proteins related to oxidative stress, reproduction and endocrine disruption were analyzed. Results indicated no mortalities in exposed groups compared to unexposed individuals. The growth of organisms was significantly affected in individuals exposed to water samples taken during the event near points of discharge compared to further down the river. The number of neonates produced per exposed individual compared to controls was significantly impacted at the most polluted site, showing both decrease and increase of the reproductive output during and after the discharge, respectively, suggesting possible reproductive impairment. Quantitative RT-PCR analyses of candidate genes and enzyme activity/protein content measurement helped to identify the modes of action underlying the physiological effects observed. Overall, present results contribute to a better understanding of the metal contamination and biological impacts associated with untreated municipal wastewater discharges on the aquatic environment.

WP124 Is altered performance and behavior linked to energetic biomarkers in a freshwater snail exposed wastewater treatment facility effluent?

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Municipal wastewater treatment facility (WWTF) effluent is a point-source for environmental toxicants entering aquatic ecosystems. Due to incomplete wastewater treatment practices, WWTF effluent contains a plethora of toxicants, such as synthetic estrogens, pharmaceuticals, toxic metals, and pesticides. Typically, these contaminants are not present in WWTF effluent at high enough concentrations to cause direct mortality. Nonetheless, exposure to contaminants may be energetically demanding to the organism in two ways: (1) Toxicants can impair the organism's ability to acquire and mobilize energy. (2) Detoxification can increase energy allocation to tissue maintenance, and consequently reduce the energy available for other biological processes (e.g., reproduction, growth, activity). Toxicant-induced changes in energy allocation may affect an organism's performance capacity and their ability to perform ecosystem services. In this study, we were interested in whether molecular energetic-biomarkers are associated with whole-animal performance. We exposed snails (*Helisoma trivolvis*) to whole effluent (0%, 12 %, 25%, 50%, 100%) for 28 days and measured growth, egg production, shell-crush resistance, activity, latency to emerge from shell, and crawl-out behavior. Additionally, we generated novel sequence data for genes involved in energy regulation at the whole-animal (molluscan insulin-like peptide) and cellular (AMP-activated protein kinase) levels. We quantified relative expression of these genes via RT-qPCR. Effluent exposure altered egg production, crush resistance, and several behaviors, and these effects appear to be linked to energetic biomarkers.

WP125 Wastewater pollution as a potential limiting factor for salmon recovery

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Wastewater pollution is a growing concern for aquatic habitat quality throughout the Pacific Northwest. The environmental health consequence of wastewater effluent for salmon, in particular, is largely unknown. Water free of toxic chemical contaminants may be critical for the recovery and productivity of salmon populations currently experiencing historically low population abundances. Current wastewater effluent criteria for contaminants in surface waters (i.e., aquatic life criteria) are established thresholds for the protection of aquatic life based on water quality standards developed and revised by the U.S. Environmental Protection Agency (EPA) to accurately reflect the latest scientific knowledge as mandated in the Clean Water Act (CWA) of 1972. A limited number of individual chemical compounds are covered; these include conventional pollutants (e.g., pH, biochemical oxygen demand, etc), priority pollutants, and non-conventional pollutants. In the U.S., the EPA mandates that permitted effluent meet water quality standards for the protection of aquatic life, but there are exceptions, including: (1) water quality standards are lacking for nearly all contaminants of emerging concern, including pharmaceuticals and personal care products (e.g., estrogenic chemicals, microplastics in toothpaste and skin care products), therefore these are not monitored; and (2) discharged compounds can be above established thresholds for the protection of aquatic life in permitted mixing zones. This review describes a screening-level assessment focused on wastewater effluent with a specific focus on salmon health and survival, including species differences, critical life stages, life history considerations, and unique vulnerability due to migration corridor length. The goal is to better understand the extent to which wastewater may be a limiting factor for salmon recovery.

WP126 Wastewater Effluent and Surface Water Quality and Quantity Trends in the United States

S. Siddiqui, J.L. Conkle, Texas A&M University Corpus Christi / Physical and Environmental Sciencees

Concerns over water quantity and quality are increasing due to population growth and climate change. Resource stress will also be compounded by the urban water cycle, where water is extracted, used, treated to regulatory requirements and typically discharged into nearby surface waters. The receiving waters must be able to assimilate this lower quality water, which could lead to problems when water volumes decline as populations grow and climate change intensifies. Therefore, wastewater treatment facilities could have to treat larger volumes (due to population growth) to higher standards before discharge. Since 2012, the USEPA has published wastewater discharge volumes, in addition to DO, pH, Nitrogen as ammonia, BOD and many others online at the EPA's ECHO website. This data will be compared with surface water data (from USGS) over the same period for major cities in the U.S. with varying population trends across multiple climate regions (arid, semi-arid, humid sub-tropical, etc.), in an effort to assess the relationship between surface water quality, wastewater effluent, population growth and climate change. This analysis will provide short-term trends due to this newly available data, but will also serve as a foundation to assess the urban water cycle's influence on contaminants of emerging concern in surface waters and food production.

PAH Mixtures in the Environment – Identifying Sources and Assessing Risks

WP127 Characterizing baseline fish health of and risk posed by oil spills to pallid sturgeon (Scaphirhynchus albus) in the Upper Missouri River Basin

D.R. Rouse, US Fish and Wildlife Service; D.E. Tillitt, USGS / Columbia Environmental Research Center; K.J. Nelson, US Fish and Wildlife Service / Environmental Contaminants Program

In Montana and North Dakota, dams have restricted the movements of a small population of adult wild endangered pallid sturgeon (*Scaphirhynchus albus*) that are estimated to number less than 100. This population is located with the Williston Basin, which includes the Bakken and Three Forks Formations that have at times produced more than 1 million barrels of oil per day. Exposure to polycyclic aromatic hydrocarbons can illicit adverse outcomes in fishes, including immune and reproductive function, with the potential for population-level consequences. Multiple oil spills have occurred in this area which threaten pallid sturgeon and their habitat and the potential for future spills is great due to enhanced movement of oil in this region. Therefore, one objective of this study is to conduct a pallid sturgeon risk assessment to characterize the risk posed by oil spills to this population of fish. Baseline data of fish health assessment metrics on pallid sturgeon or other surrogate species for proper interpretation of post-spill monitoring data has never been collected in this region. As such, a second objective of this study will be to collect baseline fish health assessment metrics for pallid sturgeon and other surrogate species under the adverse outcome pathway framework that will include changes in gene expression in various tissues, differential changes in white blood cells, polycyclic aromatic hydrocarbon DNA-adduct formation, histological changes in gills, metabolic oxidative stress, immune dysfunction, and disruption of endocrine signaling pathways. Results from the risk assessment will allow for focused oil spill response planning within this region where areas of high risk are identified and the baseline fish health metric data will be incorporated into response plans to be used during post-spill monitoring.

WP128 EACs in the Environment: Freshwater and Saltwater Pond Remediation in Bermuda

D.J. Fort, M. Mathis, Fort Environmental Laboratories, Inc.; J.P. Bacon, Bermuda Zoological Society

Indications of endocrine disruption and reproductive impairment in cane toads, killifish, and, diamondback terrapins in freshwater and marine ponds in Bermuda have prompted efforts to remediate polyaromatic hydrocarbons (PAH) and oxygenated PAHs (OPAH) within the pond sediments. A modified Tier 1 EDSP 21-day Fish Short-Term Reproduction Assay (FSTRA) was used to evaluate the effects of sediment exposure from freshwater and saltwater ponds in Bermuda on reproductive fecundity and endocrine function in fathead minnow (*Pimephales promelas*) and killifish (*Fundulus heteroclitus*), as well as, the effectiveness of remediation strategies. Baseline modified FSTRA assessment showed decreased reproductive fecundity, lower male body weight, and altered endocrinological measures of reproductive status were observed in both species. Higher plasma T levels in female minnows and 11-KT levels in both male and female minnows and female killifish exposed to freshwater and marine sediments, respectively. Decreased female E2 and VTG levels and gonadal CYP 19 (aromatase) activity were also found in sediment exposed females from both species. Solar powered, diffuse, small bubble aeration was installed within one freshwater, and subsequently, in one saltwater pond. PAH, OPAH, and anaerobic degradation product levels were monitored over the course of 3 years and post-remediation modified FSTRA was used to assess remediation status of the freshwater and saltwater ponds. Reproductive performance and endocrine endpoints were similar to control sediment levels indicative of successful remediation.

WP129 Indoor levels of polycyclic aromatic hydrocarbons (PAHs) and phthalate esters (PAEs) in participants from the Kingston Allergy Birth Cohort (KABC)

Y. Wan, University of Toronto Scarborough / Physical and Environmental Sciences; M.L. North, A.K. Ellis, Queens University / Biomedical and Molecular Sciences; M.L. Diamond, University of Toronto / Earth Sciences

Canadian children are widely exposed to semi-volatile organic compounds such as polycyclic aromatic hydrocarbons (PAHs) and phthalate esters (PAEs) from indoor environments. Both sets of compounds have been implicated in allergic diseases such as asthma. Herein we characterize indoor concentrations and estimate exposure of young children to PAHs and PAEs enrolled in the Kingston Allergy Birth Cohort (KABC). We collected floor dust samples in the bedrooms of 79 the children, living in 50 homes, and additionally sampled the most used room in each home. Dust samples were analyzed for 16 PAHs and 8 PAEs. PAEs and PAHs were commonly detected in the KABC homes, occurring over a wide range of concentrations. Questionnaire data was also collected on participants' activities and environmental/household characteristics, and correlated to indoor concentrations of PAHs and PAEs. The exposure of young children to PAEs and PAHs via dust ingestion was also estimated and will be discussed.

WP130 Tracking PAH Sources to the Gowanus Canal Superfund Site

T. Saba, Exponent, Inc.; L. Cook, Exponent; N. Johnson, Exponent, Inc.; J. Pietari, Exponent, Inc. / Environmental Sciences; P.D. Boehm, Exponent, Inc. / Environmental & EcoScience Group

Since its creation in the late 1800s, the Gowanus Canal, a narrow industrial waterway in Brooklyn NY, has been receiving solids from combined sewer overflows (CSOs) and industrial discharges (e.g., petroleum terminals, coal yards, tar distillation operations, manufactured gas plants, incinerators, and ship building and repair). These discharges resulted in Canal pollution that was documented in newspaper articles, historical and public health reports since the early 1900s. In 1911, in response to the concerns over water quality, the Gowanus Canal Flushing Tunnel, connecting the head of the Canal to Buttermilk Channel was constructed by New York City to flush sewage and offensive industrial contaminants out of the Canal and to enhance the water quality. However, this Flushing Tunnel was not able to flush the contaminated sediments out of the Canal, but resulted in mixing the Canal sediment, with the contaminants therein. Industrial and CSO discharges continued to occur for a century, even after the 1970s evolution of environmental regulations. In 2013, the Canal became a Superfund site with PAHs implicated as one of the chemicals of concern. Tracking PAH sources to the Canal sediment from ongoing and historical operations had an implication on remedial cost allocation. The complexity of the Canal setup required the use of multiple lines of chemical forensic tools, not only for PAHs, but also for other COCs and for industrial chemical tracer to ultimately track PAH sources. This presentation describes the tools used to reconstruct the historical and ongoing PAH discharges to the Canal. The analyses that will be discussed include a) the use of CSO markers and industrial tracers to track the co-discharged PAHs, b) PAH fingerprinting tools (e.g., PAH profiles, diagnostic ratios, PCA), and c) the utilization of information about other COCs to assist in tracking PAH sources.

Environmental Chemistry

WP131 A rapid screening method for pathogenic bacteria by using chemical contaminants at Triathlon swimming courses in Tokyo bay

Y. Kameda, Chiba Institute of Technology / Creative Engineering

The coastal areas are important for sound ecosystems and economic activities such as fishing industries and recreations. Especially, Triathlon games will be held at coastal areas in Tokyo bay Odaiba marine park) in 2020 Tokyo Olympic game. However, surface water in swimming areas for triathlon is contaminated by chemicals and pathogens in combined sewage overflows (CSOs) from Kanto metropolitan area. Occasionally, concentrations of *Escherichia coli*(*E. coli*)exceed the water quality standards recommended by International Triathlon Union (ITU). Therefore, effective countermeasures should be performed by 2020 Olympic games and water quality should be monitored during the games. But, it takes about one day to measure the concentrations of these bacteria by general methods. Moreover, these concentrations fluctuate daily by weather condition. As a result, it is difficult to evaluate these concentrations and human risk on the day of the games. Adding to that, CSOs also carry chemical pollutants, especially fecal sterols. Though these contaminants do not have adverse effects on athletes, previous reports discussed their correlations to *E. coli*.This study will demonstrate some simple indicators for *E. coli* at swimming courses in Tokyo Bay. The concentrations of these pathogenic bacteria are monitored with simple water qualities such as transparency, suspended matters (SS), total organic carbons (TOCs), caffeine and crotamiton. Transparency and SS are influenced intensely by algae bloom in the bay. However, a new TOC analyzer could demonstrate water contamination near coastal areas and could reveal that dissolved organic carbon concentrations have a strong relationship to *E. coli* concentrations at Tokyo bay. This may be caused by various unknown fecal pollutants in dissolved phase. Caffeine and crotamiton are reported to be useful indicators for contamination by sewage water. This study also revealed their relation to *E. coli* concentrations. A battery of TOCs and several chemical pollutants has potential about simple and rapid indicators for pathogenic contamination and human risk at swimming courses. We also develop a smartphone software application that allows for on-site estimation of water quality as well as risk evaluation for swimmers in Odaiba marine park. Furthermore, we try to establish novel water monitoring and swimmers risk management procedures (including smartphone software) for recreational water area near metropolitan area.

WP132 The development of a passive sampling device to evaluate dissolved oxygen concentration in benthic zone

Y. Kameda, Chiba Institute of Technology / Creative Engineering

The monitoring of the concentration of dissolved oxygen is very important to keep a broad spectrum of benthic organisms as well as rich ecosystem and sound behaviour of nutrients and pollutants in lakes and the sea. In Japan, dissolved oxygen is one of water quality standards in lakes and bays. However, it is very hard work to monitor the DO concentration frequently at sampling sites and evaluate the horizonal distribution. Do sensors are very useful but very expensive. The grab sampling method is less expensive than monitoring by senssors but the data is snapshot data. The DO monitoring will need continuous data for benthic organisms because the aquatic environment in benthic zone is heterogeneous spatiotemporally. This presentation shows a novel moinitoring techique to measure DO concentration in benthic zone in lakes and the sea by using a passive sampler. The passive sampler consists of chemcatcher holder, absorbent film and diffusion limiting membrane. The color of the filter chenges accxording to the absorbed amount of DO. Therefore the absorbed amount is estimeted from the color. The color can be qualified and quntified by low cost color difference meters. Laboratory calibration tests revealed that the absorbed amout of DO increased linerarly during the deployment. The calibration tests are revealing sampling rates and effects of flow rate and water temperrature to them. After that, this device including a special cage will be applied to lakes and the sea to compare time weighted average concentration of DO by passive sampling with continuous monitoring data by using DO sensors. This deice is very easy to operate and low cost so that it will be one of useful techniques to monitor spational distributions and temporal variation of DO concentration in benthic zone in lakes, bays and the sea.

WP133 Development of compact autonomous samplers for monitoring of micropollutant time-weighted average concentrations in particulate phases

Y. Kameda, Chiba Institute of Technology / Creative Engineering

Grab sampling is a very fundamental and effective sampling method to measure micropollutants in surface water when the “sampling frequency” is high. However, high frequency sampling is difficult in most investigations and screenings. Passive sampling techniques have great advantages to estimate time-weighted average concentration of pollutants and to their on-site accumulation during their deployment. But passive sampling techniques have also disadvantages to measuring micropollutants in particulate phases. Laborious calibration for the estimation of sampling rates of micropollutants is also needed. Recently small continuous low-level aquatic monitoring “C.L.A.M.” has been developed which can perform low flow rate extraction sampling at sampling sites. C.L.A.M. is very effective tool to estimate micropollutant concentration in low turbidity surface water. However sampling flow rates of water depend on water turbidity. As the result, it is difficult to estimate accurate time-weighted average concentration of micropollutants even in dissolved form during the deployment. Therefore it is very meaningful to develop novel samplers to monitor micropollutants automatically in particule phase even in muddy surface water for a week. In this study, a novel autonomous sampler were developed. This sampler, which is called “GRAVE” needs only two D size batteries for 9 days accumulation. The size is small, 18cm(W)x 23cm(L)x10cm(H). It is a submerged sampler which can filtrate water continuously at constant rate even in very muddy water with more than 100 mgSS/L. The flow rate ranges from 0.31 to 0.33 ml/min. Laboratory recovery tests revealed that recovery rates for Kaolin, total organic concentration (TOC), total nitrogen (TN), total phosphorous (TP) and suspended solids (SS) were 96.2%, 78.2%, 35.4%, 71.3% and 78.6%, respectively. The recovery of TN is relatively lower than other results because of high solubility. TOC contained biodegradable fractions, but relatively higher than expected. This may be caused by bacteria growth in accumulation tank. The GRAVE will be a powerful sampler to estimate time-weighted average concentration of particulate less-biodegradable compounds such as microplastics, DNA, and POPs, etc.

WP134 Evaluation of passive and active sampling technologies for PAHs in Lake Powell, AZ

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The measurement of trace concentrations of organic contaminants in natural waters can be challenging due to the volumes of water required to achieve sufficient masses of analytes for detection. In this work, we compared two techniques for measuring concentrations of polycyclic aromatic hydrocarbons (PAHs) in Lake Powell, AZ during the summer of 2016. A passive sampler, the semipermeable membrane device (SPMD) and an active sampler, the Continuous Low-Level Aquatic Monitoring (C.L.A.M.) device, were co-deployed at eight sites. The SPMDs were deployed for 28 days and the C.L.A.M. samplers were deployed for approximately 24 hours at the SPMD deployment and retrieval dates. A series of field and laboratory blanks, analyte recovery spikes, and field replicates were employed to evaluate the two sampling techniques. There were no detections of PAHs in the SPMD blanks whereas there were 5 detections in one of the C.L.A.M. blanks at concentrations comparable with the low end of the environmental concentrations. Variability between replicate SPMDs was less than that measured in replicate C.L.A.M. samplers; relative percent differences were 5-22% for the SPMD and 40-114% for the C.L.A.M. Recoveries of PAHs spiked into each sampler were greater in the SPMD than the C.L.A.M., with average recoveries of 73% and 54%, respectively, for 33 analytes. At several sites, a stacked extraction disk system was used in the C.L.A.M. to determine analyte breakthrough. Analysis of the individual disks indicated that the primary extraction disk was effective in retaining nearly all of the sampled PAHs. Estimated concentrations of PAHs in water from Lake Powell were higher with the C.L.A.M., but more compounds were detected with the SPMD. However, a greater number of the higher molecular weight PAHs were detected by the C.L.A.M.. Both sampling systems have limitations; the SPMD requires a longer (weeks to months) deployment period, increasing the possibility of damage or loss of the device whereas the C.L.A.M. can experience issues of clogging of the pre-filter and extraction disks, pump failure, and inaccurate methods of determining the volume of water extracted. Although both devices have promise in environmental monitoring, the more established SPMD was chosen for subsequent studies in Lake Powell.

WP135 Evaluation of PUF-PAS Sampling Rates in Any Environment

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Passive air samplers equipped with polyurethane foam media (PUF-PAS) are important tools for measuring atmospheric concentrations of persistent organic pollutants, but accurate and consistent determination of compound-specific effective sampling volumes for PUF-PAS deployments has been limited by the expense and uncertainty of sampler calibration and depuration approaches. Here, we evaluate global performance for compound-specific sampling volumes in a process-based hourly model using five semi-volatile depuration compounds and 82 samples collected by the Global Atmospheric Passive Sampling (GAPS) Network in 2006-2007. We evaluate the global spatial and seasonal distribution of depuration-based and simulated PUF-PAS sampling rates, quantify global predictive performance, and identify future research directions and operational approaches for refining estimates of PUF-PAS sampling rates. We also evaluate the performance of the model in an indoor environment through an independent uptake study. Results support operational use of simulated sampling rates in any environment.

WP137 Airborne PCBs and OH-PCBs inside and outside urban and rural U.S. schools

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PCBs appear in school air because many U.S. school buildings were built when PCBs were intentionally added as Aroclors to building materials and because PCBs are also present in modern pigment through inadvertent production. The presence of PCBs in schools is concerning because children are especially vulnerable to the toxic effects of PCBs. Exposure to PCBs in schools is receiving increased attention by researchers and the public and in some cases has led to lawsuits seeking testing and remediation of PCBs. However, U.S. schools are not required to test for the presence of PCBs, and few research studies measuring inhalation exposure to PCBs in schools have been published. Here we report indoor and outdoor air concentrations of PCBs and OH-PCBs from two rural schools and four urban schools, the latter near a PCB-contaminated waterway of Lake Michigan, in the United States. Samples (n=108) were collected as in/out pairs using polyurethane foam passive air samplers (PUF-PAS) from January 2012 to November 2015. Samples were analyzed using GC/MS-MS for all 209 PCBs and 72 OH-PCBs. Concentrations inside schools were one to two orders of magnitude higher than outdoors and ranged 0.5-194 ng/m^3 (PCBs) and 4-665 pg/m^3 (OH-PCBs). We believe this is the first study to report indoor OH-PCB concentrations in air. Concentrations outside ranged 0.07-3 ng/m^3 (PCBs) and below detection-18 pg/m^3 (OH-PCBs). PCB congener profiles were similar within each sampling location across season but different between schools and indicated the sources as Aroclors from building materials and individual PCBs associated with modern pigment. Humans are readily exposed to PCBs through diet, inhalation, and dermal contact, with diet long-identified as the major exposure route. Inhalation exposure in our cohort was determined to be as high as 115 and 116 ug/yr for girls and boys, respectively, compared to dietary exposure of 66-108 ug/yr for girls and boys, respectively, in the same cohort. This study is the first cohort-specific analysis to show that some children's PCB inhalation exposure may be equal to or higher than their exposure through diet.

WP138 An approach to reduce uncertainty in ecological hazard assessments associated with Aroclor data

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Aroclor data are frequently the only data available for environmental matrices because it's cost effectiveness and historical significance at many contaminated sites. Evaluation of toxicity based on Aroclor data has historically relied on concentrations of individual Aroclor mixtures, or summation of Aroclors to represent total PCBs, as the causative agent for toxicity and are thus, uncertain. One approach to reduce the uncertainty associated with these data in risk assessments is to translate reported Aroclor concentrations to PCB congener specific concentration data, specifically dioxin-like (DL) PCB congeners. Knowing the concentration of these DL-PCB congeners can reduce uncertainty in evaluating adverse effects to wildlife because dioxin-like toxicity has been mechanistically associated with the most sensitive ecotoxicological endpoints. However, it understood that translating Aroclor data to congener data using conversion factors based on pure mixtures also involves a degree of uncertainty due to the unknown impact of environmental weathering on the these complex mixtures. To address these questions, a carp study was conducted in 2011 on the Lower Fox River (LFR) to create a matrix of information that could be used to test the accuracy of converting Aroclor congener data. Specifically, this study measured Aroclor, PCB homolog, and PCB congener concentrations in carp such that measured and predicted values could be compared on fish specific basis. Results from this study indicated that: (1) with exception of MonoCB, DiCB, and DecaCB homologs, median relative percent differs for the other homolog groups ranged from 5.8 to 55% with difference generally being less than 1.5-fold, (2) Median relative percent differences between measured and predicted dioxin-like PCB congener concentrations ranged from 16 to 73% with differences being generally being less than 2-fold. (3) PCB126 was the greatest contributor to mammalian TEQs while PCB77 was the greatest contributor to avian-TEQs in carp (5) The median relative percent differences for total TEQs were 54% and 30% for mammalian and avian TEQs, most differences were less than 2-fold. Overall, the results from these analyses show that in nearly all instance, predicted DL-PCB congener concentrations were reasonable approximations of measured values in this study.

WP139 PCB fingerprinting in biota and scat

L.A. Rodenburg, Rutgers University / Environmental Sciences

Fingerprinting of PCB congener patterns in air, water, and sediment has helped us identify PCB sources in ecosystems across the United States, where all 209 PCB congeners are routinely measured. In these environmental compartments, PCBs from Aroclors retain identifiable fingerprints. In biota, however, fingerprints are more altered, so identifying the PCB source can be challenging. This presentation will discuss the results from fingerprinting of PCB data from both freshwater (Portland Harbor Superfund Site and the Hanford Site, WA) and estuarine systems (the Duwamish River near Seattle, WA) and across a variety of organisms, including benthic organisms, crabs, fish, osprey eggs, and otter scat. Across these varied species and ecosystems, there are common fingerprints that are similar to Aroclors but have undergone weathering via characteristic ADME processes. Co-located sampling demonstrates that spatial patterns of contamination are consistent in both sediment and benthic biota. Otter scat shows unique congeners patterns which suggest that the alteration of PCB fingerprints in biota is dominated by metabolism, not excretion.

WP140 Legacy Contaminant Fate in the Gulf of Mexico

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The Mississippi river and its tributaries pass through 31 states, and ultimately empty into the Gulf of Mexico, with it many legacy chlorinated hydrocarbon contaminants. Legacy contaminants such as PCBs and DDT have been banned for over 30 years but often remain in the environment, as do their degradation products. These contaminants enter the environment through runoff from agricultural lands, the destruction/disposal of industrial plants and equipment, from emissions from construction materials, and old electrical equipment. Sediment samples from five locations were collected during a NSF cruise from 26 August 2016-7 September 2016 using a multicore collection method. Samples were taken along a transect extending from the mouth of the Mississippi River to a deep offshore location where no contaminants would be expected to be found. Samples were divided into subsamples by being cut into twelve 1 cm slices. Chlorinated hydrocarbons will persist in sediments however little information has been generated about their presence in lower Mississippi River sediments or coastal sediments near the mouth of the Mississippi River. Accelerated Solvent Extraction and Gas Chromatography-Mass Spectrometry will be used to detect and quantify legacy contaminants amounts in sediment core samples collected at the 5 locations within the Gulf of Mexico.

WP141 Identifying sources of toxics using biofilms

W. Hobbs, Washington State Department of Ecology / Environmental Assessment; S. Collyard, C. Larson, Washington State Department of Ecology

Biofilms (algae, microbial biomass, and organic detritus) growing on rocks, contribute to the base of the food web in rivers and streams. In contaminated waterbodies biofilms take up and bind contaminants from the water. Over the last few years we have been using the contaminant concentrations of biofilms to assess the spatial distribution of metals and organic contaminants. We have also been measuring the bioconcentration of toxics from the water to assess the accumulation in higher trophic levels. In this presentation we will provide an overview of our work in Washington State assessing metals impacts from a former mine complex and legacy organics (PCBs and DDT) in a large high-energy river. Concentrations of organics in the water column were measured using passive samplers (semi-permeable membrane devices) during various flow regimes. Our results show a strong, statistically significant relationship between dissolved concentrations of PCBs and DDT in the water column and the concentrations bound in and on biofilms. We find metal concentrations in biofilms to be a correlated with changes in periphyton community structure in addition to being a more sensitive sample media of metal contamination than water and sediment. Lastly, because biofilms represent an entry into the food web we are able to relate the contaminant source to the accumulation of organics in resident fish. The use of biofilms in source identification studies has proven to be highly effective under a number of hydrologic conditions and for various toxic contaminants.

WP142 Effect of sample mass on percent recovery of a low-polarity persistent organic pollutant fraction in chicken fat

R. Cooper, Arkansas State University; J.L. Bouldin, Arkansas State University / Biological Sciences

Obtaining large samples of animal tissue for analytical analyses while avoiding adversely-affecting sampled organisms has always been a challenge for biologists and chemists. Larger samples tend to yield better results, but also increase the risk for the organism being sampled. Therefore, determining the smallest sample that can still provide the best analytical results without sacrificing organism health is important to furthering conservation goals. In this study, organic chicken fat was used as a surrogate for wild bird fat, and masses ranging from 0.01-1.00 g were fortified at 10 µg/L and 25 µg/L with a low-polarity persistent organic pollutant (POP) fraction containing PCBs (congeners 138, 153, 180) and *p,p'*-DDE. Percent recovery of all analytes decreased with decreasing fat mass, but most remained within the 70-120% acceptable recovery range. Therefore, the evaluated method is suitable for use in studies monitoring low-polarity POPs in small quantities of avian fat over a range of sample masses.

WP143 Estimation of the Adsorption Coefficient (Koc) of Pendimethalin in Soil and Sewage Sludge Using High Performance Liquid Chromatography (HPLC)

A. Raithatha, Jai Research Foundation / Environmental Fate & Metabolism; R. Chauhan, Jai Research Foundation / Environmental Fate and Metabolism; N. Khan, Jai Research Foundation / Chemistry

Adsorption co-efficient (K_{oc}) is the ratio between the concentration of the substance in the soil/sludge and the concentration of the substance in the aqueous phase at adsorption equilibrium. It is a very important input parameter for estimating environmental distribution and binding capacity of a chemical with the soil organic matter and sewage sludge. It also allows the comparison of mobility between two different substances. Adsorption co-efficient (K_{oc}) of strongly hydrophobic organic substance in aqueous systems is difficult due to their very low water solubility and the potential to get adsorbed on container walls. In this study, the adsorption coefficient of Pendimethalin was determined using six different reference substances with known K_{oc} value as per the OECD guideline 121. Analytical method was developed in such a way that, at least one reference substance is above and another one is below the expected value of test compound. Out of the six Reference Standards, phenol, atrazine, linuron, naphthalene, and phenanthrene were selected from batch equilibrium data as per the OECD Guidelines N° 121. Lambda-cyhalothrin, though not recommended by the guideline, was selected based on its k_{oc} value.

WP144 Determination of residual characteristics and risk assessment on cyazofamid and its metabolite CCIM in some leafy vegetables

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This study was carried out to investigate residual characteristics and risk assessment of cyazofamid in some leafy vegetables, such as water dropwort, Korean cabbage, shallot and spinach called minor crop in Korea. The test pesticide was systemic fungicide cyazofamid 10% SC and it was sprayed three times with 7 days interval 0, 3, 7 and 14 days before harvest onto the test crops. Limits of quantitation (LOQ) was 0.02 mg/kg. Their recoveries at two fortification levels, 10 and 50 times of LOQ, ranged from 72.5 to 97.5% indicating proper analysis methods. Highest residues of cyazofamid including its metabolite CCIM were 3.79 in water dropwort, 7.86 in Korean cabbage, 1.05 in shallot and 5.04 mg/kg in spinach. Amount of pesticide residues were found to be high for close spraying samples at harvest day. Amount of pesticide residue appeared the highest in Korean cabbage and those was the lowest in shallot. This results indicated that major factor affecting residue was specific surface area on the crop. Also, considering properties of crop surface and adhesive, highest residue was Korean cabbage which has fuzz on its surface. Estimate daily intakes of cyazofamid in test crops were less than 0.6% of its acceptable daily intake, representing that residue levels of the pesticides detected do not pose an immediate health risk.

WP145 Analysis of the Urban Fate and Distribution of Fipronil following Residential Application

Z. Cryder, University of California, Riverside / Environmental Sciences; L. Greenberg, University of California, Riverside / Entomology; J. Richards, M. McGinnis, J. Gan, University of California, Riverside / Environmental Sciences

Fipronil is a phenylpyrazole insecticide commonly used in urban environments for the control of pest organisms, including ants and termites. The climate of Southern California results in the existence of pest pressures throughout the year, necessitating high pesticide use rates. Frequent applications of pesticide formulations containing fipronil as active ingredient have resulted in contamination of surface water following urban runoff of fipronil and its primary degradates (collectively: fiproles). It has been discovered that fiproles are detected in surface water at concentrations exceeding toxicity thresholds for several aquatic invertebrates. Urban runoff mitigation is therefore essential to avoid adverse effects in sensitive non-target organisms and subsequent impacts to local ecosystems. To direct runoff mitigation efforts, it is first necessary to understand the fate and distribution of fiproles in urban environments following real pesticide applications. Adsorption isotherms were constructed in the laboratory to provide a preliminary look at sorption behavior in soil, urban dust, and on concrete. Five homes in Riverside, California then received a fipronil pesticide application in July 2016 per the product label instructions: a spray formulation was applied one foot up the wall and one foot out from the wall around the entire perimeter of each home. At five time points during the period July-December 2016, runoff water, soil, urban dust, and concrete wipe samples were collected from each home. The data reveal that fiproles can persist over the period studied in soil, urban dust, and on concrete and that detectable concentrations of fiproles continue to be present in runoff water. The concentrations detected in runoff water were plotted against the concentrations found in the solid media to determine if any correlations existed. Such correlative relationships both provide a way to easily predict runoff concentrations based upon linear regression equations and reveal the sources of urban runoff.

WP146 Irradiated water sediment studies: Approaches to higher-tier work

S. Penketh, Envigo / Environmental Fate and Metabolism; A. Crowe, Envigo Research, Limited / Metabolism

Higher tier environmental fate studies are more commonly required in today's increasingly complex regulatory framework. Where photolysis is an important route of degradation the irradiated laboratory water/sediment study presents a more realistic scenario for studying the environmental fate of chemicals than the standard individual aqueous photolysis (OECD 316) and water/sediment (OECD 308) studies, and it may provide options for refining risk assessments relating to photolysis products. This study is included as an optional study for EU crop protection chemicals under Regulation (EC) No 1107/2009. Using radiolabelled test item allows the rate and route of dissipation and transformation of the test item in the system to be followed. Using natural sediment systems means that more realistic levels of the test item and its degradates can be observed in each aqueous and sediment phase. We will present the results from studies conducted using a radiolabelled crop protection chemical in natural sediment systems. The initial aquatic sediment and aqueous photolysis studies showed that, as little degradation occurred in the sediment system, photolysis would be a major route of degradation in the environment. Therefore an irradiated water/sediment study was conducted to provide more realistic results and allow refinement of the risk assessment. In the standard studies, although the rate of dissipation from the water phase was reasonable, the rate of degradation in the total aquatic sediment system was very slow while the rate of photolytic degradation under natural summer sunlight at latitude 40°N was extremely rapid. The parent chemical was photolysed to several degradates >10% including small polar components and carbon dioxide. In the irradiated water/sediment study DT50 values obtained in the water phase (dissipation) and total aquatic sediment system (degradation) were very much lower. In addition, the same degradates as those formed in the aqueous photolysis study were found in the sediment and water phases but at lower overall levels.

WP147 The Aerobic Mineralisation in Surface Water (OECD 309) test

S. Penketh, Envigo / Environmental Fate and Metabolism; A. Crowe, Envigo Research, Limited / Metabolism

The Aerobic Mineralisation in Surface Water (OECD 309) test is now an established data requirement under Regulation (EC) No 1107/2009 for crop protection chemicals and is also being used for testing chemicals under REACH Regulation (EC) No 1907/2006. The OECD 309 test is designed to assess the fate of a chemical applied to aerobic natural surface water. The principle objective of the OECD 309 study is to determine the rate of mineralisation to form carbon dioxode; this is assessed using low concentrations of the chemical to ensure that the biodegradation kinetics obtained reflect those expected in the natural environment. In our laboratories we have utilised different test systems (pelagic and sediment amended, river and lake water) and experimental designs (air flow-through and static, dark and irradiated incubation conditions) over a number of years in an attempt to optimise the outcome of these studies and meet a variety of experimental challenges. The challenges faced have included selection of test concentrations, obtaining acceptable mass balance of radioactivity, formation of biofilms and detection and identification of degradation products. This poster presents a comparison of the different test systems and study designs based on testing a range of crop protection products.

WP148 Separation and analysis of harmful fungi using magnetic nanoparticles

D. Kang, J. Lee, J. Shin, Kyung Hee University; E. Cho, S. Yang, Kyung Hee University / Applied Chemistry

Airborne fungi have many impacts on our indoor environment. They can cause asthma, rhinitis, sinusitis, atopy, pneumonia, sick building syndrome. Several groups reported the relationship between indoor airborne fungi and respiratory disease. In particular, some fungal species emit toxins which can cause severe health effects. For example, Ochratoxin produced by Aspergillus ochraceus and Penicillium viridicatum can affect impairment of kidney function, blood in the urine and necrosis of lymph nodes and fatty liver changes. Therefore, it is highly desirable to develop a convenient and rapid detection method to separate and determine the fungi concentration. In the present study, we have synthesized silica coated Iron oxide nanoparticles (Fe_3O_4@SiO_2-COOH) and modified the surface with specific fungi's antibody using peptide bond to separate the target fungus from fungi mixture. After separation the desired fungus, we carried out the XTT assay to determine the concentration of separated fungus. We believe that the results can apply to real-time monitoring of harmful fungi in the indoor environment.

WP149 Ethynylestradiol in wastewater influent, effluent, and U.S. streams (2011-2016)

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The synthetic steroid hormone, 17alpha-ethynylestradiol (EE2), has been used in birth control pills in the United States (US) for more than 50 years. EE2 often is used as the primary active estrogenic component in many formulations of combined oral contraceptives or contraceptive patches, which typically also include a progestin. EE2 has other less common pharmaceutical applications. EE2 is eliminated from the body in both feces and urine. As such, waste-water treatment plants (WWTPs) are expected to be important source inputs of EE2 to streams and other water resources, either from direct effluent discharge or from rain or irrigation-induced run-off following the application of WWTP biosolids to agricultural fields. EE2 is recognized as a potent estrogen and, thus, a possible important chemical contaminant to consider with respect to endocrine disruption for aquatic species exposed to effluent discharges and biosolids runoff. In a variety of water-quality studies conducted from 2011-2016, the US Geological Survey collected more than 170 influent or effluent samples from WWTPs, and more than 1300 water samples from streams across the US, many of which receive direct discharges from WWTPs or indirect discharges from septic systems. Non-conjugated steroid hormones, including EE2 and estrone, were isolated from filtered or unfiltered water using solid-phase extraction. Extracts were cleaned-up with Florisil SPE, and method compounds were derivatized to trimethylsilyl analogs and analyzed by GC/MSMS using isotope dilution quantification (including EE2-d4). EE2 was detected in < 6% of either WWTP influent or effluent samples (reporting level 0.8 ng/L) and ranged up to about 4 ng/L, with most detections below 1 ng/L. Detections of EE2 were rare (< 0.3%) in stream-water samples, with concentrations < 2 ng/L. By contrast, although estrone has less estrogenic potency than EE2, it was nearly ubiquitous in WWTP influent/effluent samples, and commonly was detected in river samples that receive WWTP discharges. The EE2 concentrations observed from the large number of both stream and WWTP samples collected from many sites across the US in 2011-2016 are much lower than those previously reported (73 ng/L median, 831 ng/L maximum) for a smaller (N=70) reconnaissance study conducted in 1999-2000 by the USGS that included samples impacted by livestock production and used a different GC/MS method (Kolpin and others, 2002, http://dx.doi.org/10.1021/es011055j).

WP150 Analysis of Thyroid Hormones in Biological Samples Using Stable Isotope Dilution Liquid Chromatography-Tandem Mass Spectrometry

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Thyroid hormones (THs), which are critical for growth and development in all vertebrates, can be impacted through chemical perturbation of the hypothalamic-pituitary-thyroid (HPT)-axis. Mammalian and amphibian models are being used to address this research priority within the USEPA. Determining hormone levels in blood and tissue is essential for understanding chemical effects on the HPT axis and therefore critical for the development of thyroid-related adverse outcome pathways (AOPs). We have developed analytical methods to measure THs in blood, glands and brain using stable isotope dilution liquid chromatography-tandem mass spectrometry (LC-MS/MS). Samples from our experiments can have small tissue mass or volume, low hormone concentrations, and complex matrices (proteins, lipids). To handle this sample diversity and complexity, several sample preparation procedures are employed. Serum is hydrolyzed with acid, gland samples are enzymatically digested with protease and brains are extracted with chloroform and methanol and THs are back extracted with $CaCl_2$. Sample extracts are processed through solid phase extraction (SPE) and analyzed by LC-MS/MS. Target compounds include: 3-monoiodotyrosine (MIT), 3,5-diiodotyrosine (DIT), 3,5-diiodothyronine (3,5-T2), 3,3?-diiodothyronine (3,3?-T2), 3,3?,5-triiodothyronine (T3), 3,3?,5?-triiodothyronine (rT3) and L-thyroxine (T4). Stable isotopes, used as internal standards, include: $^{13}C_6$-3,3?-T2, $^{13}C_6$-T3 and $^{13}C_6$-T4. The instrumental analysis method is fast with chromatographic separation of 7 target compounds in 4 minutes and linear ($R^2 = 0.997$) over a concentration range of 0.01 ng/mL to 20 ng/mL. Overall the method is very sensitive and suitable for small samples (e.g., 20 µL serum, 1mg gland and 100-200 mg brain). Method lower limits of quantification (LLOQs) are 0.050 ng/mL serum, 50 pg/mg gland and 0.010 ng/g brain. This abstract does not necessarily reflect USEPA policy.

WP151 Nontarget screening of intrinsic biological chemicals and exogenous pollutants in blood of wild fish captured from Lake Tai

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With the rapid growth of the number of commercial chemicals, high-throughout analysis of numerous contaminants and identification of toxic components in environment has become an analytical technology with urgent development. In this study, a new method was developed by non-targeted screening technologies for simultaneous identification of intrinsic biological chemicals and exogenous pollutants in two different fractions in blood samples (Fraction 1: Polar fraction; Fraction 2: Non-polar fraction). Based on the combination of new data-independent acquisition (MS^e, Waters, US) scanning mode and automatic data processing by Progenesis QI (Waters, US), a full identification list was acquired including the corresponding MS/MS information of all signals with one injection. The above scanning technologies were applied to 150 blood samples from wild golden carps captured in three regions (Meiliang Bay, Zhushan Bay, and Huzhou) in Lake Tai (China), which detected 9013 peaks (1518 in F1 ESI+, 1158 in F1 ESI-, 3968 in F2 ESI+, 2369 in F2 ESI-). Searching against on-line databases including HMDB, KEGG, Lipidmaps, Massbank, Pubchem and Pubmed, 3242 compounds including 1803 compounds with corresponding MS/MS signals and 1439 compounds with only MS signals were identified, which contains 16 groups of intrinsic chemicals (phospholipids, glycerides, nutrients and etc.) and 15 groups of exogenous pollutants (pharmaceuticals, fluorine substances, surfactants and etc.). Subsequent multivariate statistical analysis was performed by Matlab (MathWorks, MA, USA) to acquire correlation coefficient of detected peaks. After that, Gephi (Gephi Consortium, USA) was applied to accomplish the network analysis of the positively related compounds (r>0.7). The network established in this study was consistent with the fish metabolism network recorded in KEGG database, which reconfirm the feasibility and accuracy of high-throughout analyzing method. Besides, the regional difference among peak intensities of intrinsic biological chemicals is likely due to the different exposure levels of exogenous pollutants, which needs further validation by in vivo exposure experiment.

WP152 Solving the challenges of conducting environmental fate studies with volatile test substances

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When conducting environmental fate studies the use of a radiolabelled test substance allows the rate and route of dissipation and transformation of the test substance to be followed. In order to achieve these objectives it is necessary to obtain a mass balance in the range of 90 to 110% of the amount of radioactivity applied. When using volatile test substances, obtaining an acceptable mass balance can be challenging. Each test substance has different properties and so will require different trapping media. In addition, if the test substance degrades, it may also be necessary to trap, quantify and identify volatile degradation products including radiolabelled methane and carbon dioxide. Different approaches to solving the problems associated with the conduct of studies using volatile test substances will be presented.

WP153 Active substances in antifouling paint released during ship's hull cleaning using water jet spray

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Since the ban on the use of organotin-based antifouling paints, many other booster biocides have been emerged to replace organotin-based products. During the maintenance of ships on a dry dock, pressurized jet water is sprayed for clearing off foulants attached on the ship's hulls. The sprayed water, which was released directly into the adjacent water column, was found to be heavily contaminated with paint particles and active substances released from the particles. Although some booster biocides are known to be easily degraded from the moment of release, they can be a source of hazard to marine organisms through continuous release from the paint particles into the environment. Paint particles accumulated in the bottom sediment also can be a continuous source of biocides and other active substances. This study is to characterize the compounds being released into the seawater through the spraying of pressurized jet water. Samples (jet water effluent, fouling organisms, paint chips, and adjacent seawater) were collected for metal and biocide analyses during ships' hull cleaning on a dry dock. Metal concentrations were determined using ICP/MS and the distribution of biocides was screened using LC Q-TOFMS. Particle size distribution was also characterized using wet sieving and laser scattering particle size analyzer. The results will give insights of compounds distributions under different particle sizes and their relative contribution to total toxic potency when released into the environment.

WP154 Environmental Depleted Uranium Analysis with ICP-MS

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Depleted uranium (DU) has been used by the US military for several purposes. Currently, there is widespread interest in the environmental fate of DU on the Big Island of Hawaii where DU was used to both aim tactical nuclear weapons as well as being used as an armor piercing munition. The military has reported low to non-detect levels of this DU using various radioactive material detectors. Inductively Coupled Plasma-Mass Spectrometry is much more sensitive at detecting this isotope. We will present findings from areas around Hawaii as well as background locations that would not have this element.

Epigenetic and Evolutionary Effects of Pollutants – New Challenges for Long-term Ecological Risk Assessment

WP155 Effects of early life exposure to benzo[a]pyrene (BaP) on gene expression and promoter methylation in zebrafish (Danio rerio)

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Early-life exposure to polycyclic aromatic hydrocarbons (PAHs) is known to cause persistent changes to patterns of DNA methylation in zebrafish (*Danio rerio*). We are interested in how these epigenetic changes affect overall health and the response to re-exposure later in life. Zebrafish embryos were exposed to the model PAH, benzo[*a*]pyrene (BaP) at concentrations of 1, 10 and 100 µg/L from ≤4 hours post fertilization (hpf) to 120 hpf. Water spiked with BaP was renewed every 24 hours. The survival, hatch success, and incidence of malformations were monitored daily and larvae were sacrificed at 120 hpf. The treatment did not significantly induce mortality, deformities, nor delay in hatching relative to controls. Expression of cytochrome P4501A, a biomarker of PAH exposure, was significantly induced in larvae exposed to BaP. To investigate the role of DNA methylation in this dysregulation, we used reduced representation bisulfite sequencing (RRBS) to identify differentially methylated regions within the genome of control and 100 µg/L BaP exposed larvae. In future work we will assess the role of differentially methylated genes in mediating sensitivity to PAHs in naïve and pre-exposed fish. These data may give us information about the potential consequences of early-life exposure of fish to contaminated sediments and will generate candidate epigenetic biomarkers for our studies with wild fish.

WP156 Lead-induced transgenerational neurobehavioral toxicity and transcriptomic changes in zebrafish brains

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Lead (Pb) is a major public health hazard in urban areas and has profound developmental and behavioral implications into adulthood and across generations. The significance of adverse health outcomes due to lead exposure is exemplified by the recent discovery of lead in municipal water systems, notably in Flint, MI. Understanding the long-term repercussions of lead exposure is crucial for elucidating the mechanism(s) of effect and heritability. For this study, zebrafish embryos (Danio rerio) are an ideal model for translational and transgenerational research due to their short generation time, sequenced genome, and analogy to human development and disease. The exposed F_0 generation was raised to adulthood, underwent neurobehavioral analysis, and was spawned to produce the F_1 generation, which was subsequently spawned to produce the F_2 generation. The behavioral testing targeted learning and memory. The unexposed F_2 generation also underwent behavioral testing and brain tissue was collected and formalin fixed for gene expression analysis. RNA was extracted from the control and 10 uM Pb-lineage F_2 generation (n=11 for each group). We prepared 3' mRNA-Seq libraries (QuantSeq) that were sequenced and the transcript expression was quantified. In the behavioral assays, both the F_0 and F_2 generations had significant learning deficits and behavioral changes. In the F_2 generation brain tissue, 367 genes were found to be significantly differentially expressed between the control and the F2 offspring of the lead exposed fish. A subset of these altered genes are known to be associated with learning and memory and also have been reported to be affected by lead exposure. These include GSK3B, EGR1, HPRT1, RHO and MAPK1. Genes associated with the endocrine system and hormone regulation (LH, FSH) also had differential expression. Pathway analysis revealed altered genes in pathways involved in neurological disorders, nervous system development and function, steroid metabolism, learning, memory and behavior. These data will inform future investigations to elucidate the mechanism of adult-onset and transgenerational health effects of lead exposure.

WP157 Transgenerational Effects of Developmental Atrazine Exposure on Medaka Fish: Alterations in Gene Expression

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Developmental exposure to endocrine disrupting chemicals (EDC) has been associated with adverse health effects in organisms at adulthood, especially when exposure occurs at vulnerable timeframes during embryogenesis. Atrazine (ATZ) is a widely used agricultural herbicide that can also disrupt endocrine function in vertebrates. Exposure studies have indicated that ATZ affects gonadal development, meiotic activity, reproductive function, gene expression, and structural defects in the offspring of exposed organisms. We examined the transgenerational effects of embryonic atrazine exposure in medaka fish (*Oryzias latipes).* Medaka were exposed at control, 5 and 50 ug/L atrazine, or 0.05 ug/L ethinylestradiol (EE2) for 12 days from the day zero of fertilization. The window of exposure overlapped with the presumed period of epigenetic reprogramming of germ cells during gonadal sex determination. Endpoints measured were fecundity, sperm count, sperm motility, fertilization success as an indicator of male fertility, and expression of genes involved in reproduction. Gene expression levels were quantified in the first generation (F0) and third generation (F2) ovary samples by real-time PCR and the expression data has been presented as fold change difference against control in each generation. Egg production was not altered; however, sperm count and fertilization success of the male fish were significantly reduced in the F2 generation. Expression of most of the genes in the reproductive axis remained significantly elevated at F0 generation followed by significant reduction of DNA methylation specific gene *Dnmt1*, whereas expression of *Cyp19a1a* was significantly elevated in both F0 and F2 generations. Measurement of differential expression of brain genes is currently in progress. Present results from the laboratory exposure study demonstrate that environmentally relevant concentration of atrazine can induce transgenerational reproductive phenotypes in the males and molecular alterations in the females.

WP158 Adaptation to xenobiotics and assessment of multigenerational effects: Applying evolutionary ecotoxicology to Daphnia magna

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Ecotoxicology has come far in recent decades in quantifying risk of chemicals to non-target organisms. A black box, however, still remains when it comes to compensatory responses and transgenerational transmission of such modifications. How do we incorporate this in our ecological risk assessments? Do such adjustments mean that organisms are free of risk as long as they are capable of establishing compensatory responses? Or do we need to consider trade-offs, like decreased fitness, when we evaluate risk? Finally, if some effects are not visible until the next generation, how do we best investigate such aspects? These questions are complicated and before we are able to answer them and make valid experimental guidelines, we need more accurate information on underlying biochemical mechanisms. For most chemicals, only one generation and possibly their number of offspring have been investigated. However it has been documented that effects can persist beyond the exposed generation. The current study follows 6 generations of parthenogenic reproduction in *Daphnia magna*. The aim is to apply evolutionary ecotoxicology to assess compensatory adjustments to the azole fungicide, prochloraz and look further into multigenerational effect of exposure. Prochloraz is a well-characterized toxicant known to both block and induce cytochrome P450 monooxidase (CYP) enzymes, which play key roles, for instance, in detoxification of xenobiotics. Resistance studies on pesticides have shown that adaptation to sublethal doses of chemicals often happens due to changes in such detoxification enzymes. CYPs are therefore our main focus in this study. The experimental design includes three treatment groups; one where all generations are exposed to prochloraz, one where only the first generation is exposed and finally a non-exposed control group. We aim to link phenotypic effects to genetic markers to better understand molecular mechanisms. The hypotheses that will be addressed are: 1) adaptive responses to the xenobiotic occur, 2) the adaptive effect will last over multiple generations and will be measurable in physiological parameters such as growth, reproduction and xenobiotic metabolic capacity measured as CYP activity 3) the adaptive effects can be explained by changed transcriptional levels of detoxification genes, which, furthermore, might be linked to heritable epigenetic mechanisms.

Canadian Oil Sands – Advancing Science in Chemical and Toxicological Characterization, Reclamation and Monitoring

WP159 The comparative toxicity of two Canadian diluted bitumens to developing yellow perch (Perca flavescens)

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With the development of the Alberta oil sands industry, the increased oil transportation through pipelines has led to the need to assess the impact of these products on aquatic organisms in smaller inland watersheds. Diluted bitumen, or dilbit, is a key product currently transported in pipelines traversing a number of Canadian watersheds, without data regarding the biological impacts on endemic fish species. Yellow perch (*Perca flavescens*), abundant in lakes and streams throughout North America, may act as ideal bioindicators representing fish species most likely to be at risk of exposure during a potential spill. We compared the chronic (16 d) toxicity of the two predominant dilbit blends currently being transported in Canada (Access Western Blend, AWB; and Cold Lake Blend, CLB) to early life stages of wild-sourced yellow perch. Embryos were exposed to dilbit through a static daily renewal of water accommodated fractions (WAF) and chemically-enhanced water accommodated fractions (CEWAF) at concentrations ranging from 0.02 – 20 µg/L total polycyclic aromatic hydrocarbons (TPAH). Hatching success and the percentage of fish showing malformations varied across treatments, with pericardial edema being the most prevalent response. As for other species exposed to dilbit, biomarkers of xenobiotic metabolism (e.g. *cyp1a*) were increased 20-fold with WAF treatment and 40-fold with CEWAF treatment. Interestingly, mRNA levels of genes involved in phase II detoxification and tumorigenesis pathways were affected in AWB CEWAF treatments, despite not reaching an EC50 for developmental malformations. We suggest that embryonic yellow perch are relatively resistant to dilbit exposure, which may be a result of impeded oil permeation in the egg due to the proteins that make up the connective strands of the egg ribbons. This is the first study to simultaneously compare the toxicity of two blends of dilbit to wild-sourced littoral zone fish.

WP160 Assessment of acute and chronic toxicity of unweathered and weathered diluted bitumen to freshwater fish and invertebrates

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This study investigates the acute and chronic toxicity of two blends of diluted bitumens ("dilbit") and weathered dilbit on freshwater fish and invertebrates after exposure to different concentrations of physically-dispersed (*water accommodated fraction*; WAF) and chemically-dispersed (chemically-enhanced WAF; CEWAF). Acute and chronic toxicity of weathered, unweathered and dispersed Access Western Blend (AWB) dilbit was evaluated on fathead minnow (*Pimephales promelas*). Acute and chronic toxicity of weathered and unweathered Cold Lake Blend (CLB) dilbit was assessed on rainbow trout (*Oncorhynchus mykiss*), and two invertebrate species, daphnia (*Daphnia magna*) and ceriodaphnia (*Ceriodaphnia dubia*). Rainbow trout exposed to unweathered CLB demonstrated a significantly higher toxicity (LC50-96h = 5.66 g/L) compared to the weathered CLB (LC50 > 18 g/L). For fathead minnow, unweathered AWB also demonstrated a significantly higher toxicity (LC50-96 h = 0.628 g/L) compared to the weathered AWB (LC50-96 h = 2.06 g/L). Chronic toxicity tests showed that fathead minnow lethality was also higher for AWB (LC50-7 d = 0.593 g/L) compared to the weathered AWB (LC50-7 d = 1.31 g/L) whereas larval growth toxicity was lower for AWB (IC25-7 d=0.312 g/L) compared to the weathered dilbit (IC25-7 d = 0.096 g/L). A lethal toxicity (LC50 = 6.43 g/L) was observed in ceriodaphnia exposed to the CLB WAF while no mortality was observed with the weathered CLB. The reproductive effects on ceriodaphnia were greater with the CLB (IC25 < 1.0) than with the weathered CLB (IC25 = 3.99 g/L). Volatile organic compounds (VOC), polycyclic aromatic hydrocarbons (PAH) and total petroleum hydrocarbons (TPH) increased as the dilbit WAF increased.

WP161 The effects on rainbow trout alevins of chronic exposures to diluted bitumens (Access Western Blend, AWB and Cold Lake Blend, CLB)

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The increased production and transportation of diluted bitumen (dilbit) from production sites to refineries, tide water, or end-users has increased the potential for oil spills in watersheds crossed by transportation corridors. Recent reports by the Royal Society of Canada (RSC) and the US National Academy of Sciences (NAS) have drawn attention to a lack of information about the behaviour and fate of dilbit in freshwater ecosystems, highlighting particular knowledge gaps in the chemical and toxicological characterization of dilbit products. Currently there are few published studies of the toxicity of dilbit to developing fish, and even fewer publications that compare the toxicity of dilbit to other oils. The chronic toxicity of AWB and CLB dilbit to rainbow trout alevins was measured with methods used previously, including tests with chemically-enhanced water accommodated fractions (CEWAF) to reduce the amount of dilbit used, and the expression of toxicity in terms of measured concentrations of oil in water. Alevins were exposed 23 d from hatch to swim up to dilutions of CEWAF, and cumulative mortality, signs of blue sac disease, and genetic responses were assessed at the end of the exposure. Dilbits were toxic to developing alevins with signs of toxicity similar to those of trout alevins chronically-exposed to conventional crude and heavy fuel oil. An exposure-dependent increase in blue sac disease and *cyp1a* gene transcription was observed following chronic AWB and CLB CEWAF exposure, as well as gene biomarkers associated with phase II detoxification, with responses indicating oxidative stress at some of the higher dilbit treatments. This research characterized the relative toxicity of AWB and CLB to rainbow trout alevins, and included novel analysis of the sensitivity of salmonids to diluted bitumen. This work also contributed to the identification of genetic biomarkers for monitoring oil spill impacts. Future comparisons of dilbit toxicity to rainbow trout with the published toxicity of light, medium, and heavy fuel oils will provide insight into the relative risk of dilbit spills to fish reproduction and recruitment.

WP162 Report on state of the science workshop on oil sand process water toxicity assessments

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Commercial production of bitumen from minable oil sands requires the use of water to transport and separate oil from the sand particles. Since mining began, the water has been stored on-site for re-use, and inventories have accumulated over time. Sustainable water management practices in the future will include return of treated mine water to the environment. The chemistry and toxicity of oil sands process-affected waters (OSPW) has been the subject of intensive study, with more than 20% of the recently published oil sands environmental research focused on the issue. The toxicity of the constituents in oil sand process water needs to be understood, and several companies have also recently participated in detailed fractionation studies focused on understanding the toxicity of OSPW. A multi-stakeholder science workshop was held in September 2017 to summarize the state of the science on toxicity of OSPW with the goal of developing practical recommendations on eventual treatment goals. This poster will summarize key outcomes from this workshop and will highlight key recommendations and research needs related to the development of water quality objectives for OSPW.

WP163 Tiered decision-making for driving long term monitoring programs in the oil sands

K.R. Munkittrick, Wilfrid Laurier University / Biology; T. Arciszewski, Alberta Energy Regulator

Monitoring and research near oil sands extraction activities has increased more than 10-fold over the last decade as interest has expanded in potential environmental impacts. Current research programs involve more than 60 Universities from 8 countries, and despite more than \$150M a year in monitoring and research investment, a minority of the results are actionable. As chemical analytical approaches become more sensitive, and as research and monitoring efforts intensify around the oil sands, the increased sensitivity and power improve our capacity to detect change. The capability to detect change needs to be balanced by consideration of potential exposure and adverse outcome pathways, potential ecological relevance, and relative risks to focus efforts. Experience obtained through 25 years of involvement with Canada's Environmental Effects Monitoring (EEM) program has shown that a tiered program informed by triggers can provide the context to make decisions about monitoring priorities. A structured, rigorous framework can be used to meaningfully inform environmental monitoring programs, and diverse types of environmental monitoring can be linked through a series of monitoring, management and forecast triggers. Understanding industry activities and processes, and decision contexts are important for orienting the research in a way that can drive change in environmental performance. This presentation will use case studies of recently published studies to evaluate an interpretation framework that could be used to focus monitoring and improve the translation of monitoring information into meaningful change.

WP164 Utilizing wood frog tadpoles and semipermeable membrane devices to monitor PACs in boreal wetlands in the oil sands region of northern Alberta, Canada

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Several recent studies have reported evidence that surface mining operations in northern Alberta's oil sands region contribute significantly to the atmospheric deposition of metals and polycyclic aromatic compounds (PACs) within the vicinity of major bitumen upgrading facilities and oil sands development. As part of the Canada-Alberta Joint Oil Sands Monitoring Plan, the present study examines the accumulation of PACs in boreal wetlands at varying distance from oil sands industrial activities with the use of semipermeable membrane devices (SPMDs) and wood frog (*Lithobates sylvaticus*) tadpoles. SPMDs were deployed in shallow lentic waterbodies adjacent to wood frog egg masses and were retrieved, along with tadpoles, approximately 35 to 40 days later. Wetlands south of the Athabasca oil sands industrial area accumulated less PACs than wetlands situated north of Fort McMurray, Alberta which were inside the oil sands surface mineable region. The highest concentrations of PACs were detected in SPMDs deployed within a 25 km radius of surface mining activity, consistent with snow deposition studies of PACs in the region. In wetlands located in the surface mineable region, PAC profiles of SPMDs and wood frog tadpoles were dominated by C1-C4 alkylated PACs, including dibenzothiophenes; these compounds represented 87-97% and 91-97% of PACs detected in SPMDs and tadpoles, respectively, which is strongly indicative of petrogenic sources. Total PACs in whole body tissue of wood frog tadpoles ranged from 111 to 195 ng g^{-1} wet weight. Contrary to differences seen in the SPMD PAC concentrations, there were no obvious differences in the sum of PAC wood frog tissue residues between wetland study sites. When individual PACs were assessed, most notably, alkylated fluorenes and dibenzothiophenes were found to be higher in tadpoles collected from a wetland located within 10 km of two bitumen upgrading facilities. The use of SPMDs in tandem with biomonitoring organisms offers considerable value when assessing the potential for exposure of aquatic organisms to PACs, however further validation is required to determine whether SPMDs can be utilized as surrogates for tadpoles for monitoring of PAC exposure.

WP165 Fish Health in the Peace River Oil Sands Area - Developing Baseline to Assess Potential Change with Development

M.E. McMaster, Environment and Climate Change Canada / Water and Science Technology Directorate, Aquatic Contaminants Research Division; G. Tetreault, Environment and Climate Change Canada / Water Science and Technology Directorate; T. Clark, Environment and Climate Change Canada / Water and Science Technology Directorate Aquatic Contaminants Research Division; C.J. Bennett, Environment and Climate Change Canada / Water Science and Technology Directorate; R. Frank, M. Evans, Environment and Climate Change Canada / Aquatic Contaminants Research Division

The Governments of Canada and Alberta developed a long term monitoring program for the Alberta Oil Sands Area initiated in 2012. As part of the expanded geographical extent of this program, we initiated studies in 2015 developing baselines for fish health within the less developed Peace River Oil Sands area. As part of the ecosytem health component of the program, we assess fish health using protocols developed through the Canadian Environmental Effects Monitoring Program for both the pulp and paper and metal mining industries. The overall design of the baseline studies are to collect three years of fish health data from a number of sites in the watershed. Sites include upstream reference areas, sites within the deposit, downstream of a municipal waste water discharge, a pulp and paper mill effluent discharge and sites further downstream. Variability in fish health endpoints and contaminant levels will be determined within and between sites in the watershed allowing for prediction of fish health with increased development. Baseline data will also be used to develop triggers of meaningful change. In this presentation we will present data for two sentinel species, the longnose sucker and the trout perch as well as contaminant data for walleye, a species used for local consumption. Endpoints include assessment of age structure, energy storage (condition, liver somatic indices) and energy utilization (growth and gonadosomatic indices).

WP166 Refining the chemical and toxicological characterization of bitumen-influenced waters

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Waters in the oil sands region near Ft. McMurray, AB can be influenced by bitumen resulting from natural and industrial sources. Ground and surface waters can traverse the natural oil sands deposit, possibly mobilizing water-soluble bitumen derived chemicals into the Athabasca River watershed. In addition to this input, recent research has identified the influence of oil sands process-affected water (OSPW) in groundwater systems surrounding tailings ponds. In the current study, a preparative fractionation protocol was applied to a sample set of bitumen-influenced groundwaters and OSPW (>150L each). The fractionation method allowed for the isolation of individual mixtures of soluble organics, from which comparisons between isolated extracts, as well as comparisons with the original source waters, were possible. Improvement of chemical characterization is under investigation through the identification of unknown entities differing between natural and OSPW-influenced waters as well as through the development of bitumen-derived soluble organic reference materials. Advancement of toxicological characterization is underway, assessing the responses from a suite of invertebrate and vertebrate bioassays using the same isolated soluble organic fraction mixtures. This work is intent on guiding more detailed fractionation studies designed to identify the compound classes of interest within the soluble organic mixtures of bitumen-influenced waters, as well as identifying the most sensitive and relevant test organisms and endpoints. This information is critical for the development of water monitoring programs in the oil sands region.

WP167 Elucidating mechanisms of toxic action of dissolved organic chemicals in oil sands process affected water (OSPW)

G. Morandi, University of Saskatchewan / Veterinary and Biomedical Sciences and Toxicology Centre; S. Wiseman, University of Lethbridge / Biology; G. Miao, Nanjing University / Environmental Sciences; X. Zhang, University of Nanjing / Biology and Environmental Sciences; J.W. Martin, University of Alberta / Analytical and Environmental Toxicology; J. Giesy, University of Saskatchewan / Veterinary and Biomedical Sciences and Toxicology Centre

Oil sands process-affected water (OSPW), produced during extraction of bitumen during surface-mining of oil sands in Alberta, Canada is acutely and chronically toxic to aquatic organisms. Organic compounds in OSPW are responsible for most toxic effects, but knowledge of the specific chemicals, or the associated mechanism(s) of toxicity, is very limited. By use of the *Escherichia coli* K-12 strain MG1655 gene reporter system, the purpose of the present study was to investigate relationships between toxic potencies, expression of genes and characterization of chemicals in each of five acutely toxic and one non-toxic extracts of OSPW. Effects on expressions of genes related to response to oxidative stress, protein stress and DNA damage were indicative of exposure to acutely toxic extracts of OSPW. Additionally, evidence presented supports a role for sulfur- and nitrogen-containing chemical classes in toxicity of extracts of OSPW.

WP168 Source identification of polycyclic aromatic compounds in snow, sediment, air and water samples from the Oil Sands Area of Alberta

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The Athabasca Oil Sands (AOS) deposits located in Northern Alberta, Canada, are considered the world's third largest oil reserve. Long-term data sets on major contaminants, such as polycyclic aromatic compounds (PACs), have been gathered in the past few years as part of the Alberta-Canada Joint Oil Sands Monitoring program, initiated in 2012. However, focus has been on a limited list of PACs. Potential sources of PACs in the AOS area include, natural erosion of geological formations, forest fires, bitumen upgrading, diesel combustion and airborne dust from roads and mining operations. This study was designed to explore PAC isomers (mainly, sulfur and nitrogen heterocyclic compounds) currently not being monitored in snowpack, lake sediment and passive air samples collected at varying distances from the main AOS developments during 2012-2015. Additionally, source samples including petroleum coke (petcoke), haul road dust and unprocessed oil sands were also analyzed. All samples were analyzed using two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC/ToF-MS). Thia-arenes and aza-arenes predominated in more than 260 well resolved peaks and were classified in at least 21 isomeric groups based on their mass spectra and their location in the 2D-chromatogram. Relative concentrations of thia-arenes and aza-arenes decreased with distance from the main developments, and with increasing depth of lake sediments, but were detected at least 50 km of the major developments. All thia-arenes and aza-arenes found in the snowpack, lake sediments and air samples, were also found in the petcoke extracts. Additionally, petcoke had a more distinct distribution compared to the road dust and unprocessed oil sands ore, but was similar to the near-field environmental samples. These results suggest that petcoke is a potential source for the identified thia-arenes and aza-arenes in near-field sites, and that these compounds have the potential to be used as source indicators for future research in the AOS area.

WP169 Selective Photolysis of Naphthenic Acids in Oil Sands Process-Affected Water

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Naphthenic acids (NA) are a complex class of aliphatic and cycloaliphatic monocarboxylic acids which are present in all natural components of petroleum. Current extraction for surface oil uses a caustic hot water extraction of petroleum distillates, producing large amounts of oil sands process-affected water (OSPW) stored in vast tailings ponds, of which runoff and groundwater leaching is a growing concern. NAs are highly persistent and present in significant concentrations in tailing waters are a primary contributor to the acute toxicity of the extracted organic acid fraction. As the Athabasca oil sands located in northern Alberta, Canada constitutes one of the largest crude oil reserves in the world, processes to reduce the concentration of NAs are of increasing concern. In this study, samples of OSPW and organic acid fractions where photolyzed to determine the efficacy of naturally occurring ultraviolet (UV-A) radiation for the reduction of NA concentrations. Photolysis of samples in a solar sunlight simulator occurred over a period of 18 days, and liquid chromatography tandem high-resolution mass spectrometry was used for selective NA detection. Preliminary results demonstrate that exposure of NAs to UV light can result in compositional changes to these mixtures, and affect NA concentrations.

WP170 Reanalysis of Snowpack and Lake Sediment Data in the Athabasca Oil Sands Region

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A number of recent publications have highlighted aerial deposition of inorganic (i.e., metals) and organic (i.e., polycyclic aromatic compounds [PAC]) contaminants in Canada's Athabasca Oilsands region. Here we provide a reanalysis of publicly available snowpack and lake sediment chemistry data collected in 2012, 2013, and 2014. Geochemical ratios from the snowpack data were used to create a series of source type-classes believed to represent major emissions source types including: oilsands mines, petroleum coke piles, upgrader stacks, and regional background. The spatial distribution of contaminant concentrations and geochemical ratios were mapped to provide further insight into their sources. Environmental loadings for these contaminants were then estimated using the source-types to determine the relative importance of different emissions sources. Finally, concentrations of these same contaminants and their geochemical ratios in lake sediments were compared to their values in the snowpack to evaluate source-receptor relationships. Results indicate a great deal of mixing among the source types, complicating their separation into distinct groups. Specific PAC ratios are significantly different among the source-type classes and are corroborated by both their spatial location with respect to known sources, and by inorganic ratios not used to classify the data (e.g., vanadium to aluminum; V/Al). When compared to snowpack data, some lake sediments indicate increases in contaminant concentrations and a simultaneous shift to geochemical ratios indicative of oilsands sources. However, many geochemical ratios in lake sediments significantly differ from snowpack values indicating complex physicochemical process likely confounding straightforward analysis of source-receptor relationships. This effort represents an independent evaluation of this publicly available data, highlights issues related to previous statistical treatments of the data, challenges some conclusions drawn from previous analysis, and provides new insights.

WP171 Mitigating Risks Associated with Oil Sands Process-Affected Waters using a Pilot-Scale Hybrid Constructed Wetland Treatment System

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Constructed wetland treatment systems (CWTSs) could provide a passive, low-energy strategy for mitigating risks associated with oil sands process-affected waters (OSPW). Over 975 million m^3 of OSPW have accumulated in the Athabasca oil sands region from bitumen extraction practices. Due to the large volumes, heterogeneous composition, and acute and chronic toxicity associated with naphthenic acids (NAs) in OSPW, passive and efficient treatment is necessary prior to discharge to decrease risks to biota in aquatic receiving systems. Hybrid components, such as solar photocatalytic reactors, can be implemented in CWTS to treat recalcitrant and toxic constituents (e.g., NAs). The research objective was to design, assemble, and measure performance of a pilot-scale hybrid CWTS for treatment of OSPW. Constituents of concern (COCs) were identified based on water quality criteria (WQC) exceedances, and included NAs, oil and grease, As, Cu, Ni, Pb, and Zn. Two replicate CWTS series were designed to promote "bulk" oxidizing conditions for aerobic degradation of NAs and oil and grease, and co-precipitation of arsenic with iron oxyhydroxides. Treatment of metals (Cu, Ni, Pb, Zn) occurred through precipitation with sulfides in reducing "micro-environments" in oxidizing wetland cells. Solar photocatalytic reactors were used as a hybrid component in the CWTS and as a "polishing" step to remove residual organics. Performance was assessed in terms of rates and extents of removal of COCs and changes in toxicity indicated by sentinel aquatic organism (*Ceriodaphnia dubia*). Mean total NAFC concentrations (as measured by Orbitrap-MS) decreased from approximately 43 mg/L in wetland inflow to 10 mg/L following a 16-d hydraulic retention time in the hybrid CWTS. Average As concentrations decreased from 0.026 mg/L in wetland inflows to 0.011-0.013 mg/L in wetland outflows but remained above the WQC of 0.005 mg/L. The mass of bioavailable metals (i.e., Cu, Ni, Pb, Zn) decreased in the CWTS; however, removal (in terms of total metal concentrations) was masked due to evaporative concentration. Compared to a laboratory control, untreated OSPW adversely affected *C. dubia* survival and reproduction. Following treatment in the hybrid CWTS, *C. dubia* toxicity (in terms of survival and reproduction) was eliminated. Results demonstrate that hybrid CWTSs can effectively decrease concentrations of NAs and alleviate toxicity associated with metal and organic COCs in OSPW.

WP172 The effects of raw and ozonated oil sands process-affected water exposure on endocrine markers and complex behaviors in zebrafish (Danio rerio)

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Oil sands process-affected water (OSPW), which is produced by the oil sands mining operations, is a complex mixture of organic and inorganic constituents that has been associated with various sublethal effects in aquatic species. Although OSPW is maintained in tailings ponds on site for water re-use there is a requirement of current regulatory approvals to eventually return treated OSPW back into the environment. To meet this objective, there is need to reduce aquatic toxicity of OSPW to ensure that there are no adverse effects on species in the receiving environment.

Degradation of the organic component of OSPW using ozone treatment is being investigated as a possible strategy to increase the quality of OSPW. There have been many studies on the effects of OSPW on fish embryos and larvae, but to date there has been relatively few studies on the potential persisting effects of OSPW exposure on later life stages. In this study, we examined the effect of developmental exposure to raw and ozonated OSPW on embryological cortisol levels and adult differences in prey capture behavior and avoidance responses to predator cues; behaviors that when deficient could impact survival outcomes in the wild. Embryological cortisol levels have been previously associated with lasting behavioral differences in individual fish. Our results suggest that prey capture efficiency and olfactory cue behavioral responses are not impaired, but instead there are shifts in typical behavioral responses observed in the exposed populations of fishes; cortisol level results are pending. The natural variability of behavioral responses as well as the swim behavior found in the control population is altered through developmental exposure to both raw and ozonated OSPW, however the impact these behavioral changes could have on survival outcomes are unknown. Regulatory guidelines put in place for the protection of fishes and their habitat are largely based on acute and chronic testing using standard apical endpoints. Complex behaviors in fishes, including feeding behaviors and predator avoidance behaviors can provide additional insight into the effectiveness of ozone treatment and will further characterize the potential impacts of OSPW exposure on fishes.

Aquatic Toxicology and Ecology – Part 2

WP173 A hardness-dependent species sensitivity distribution for nitrate

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The toxicity of nitrate to many freshwater organisms decreases with increasing water hardness. However, previous analyses have suggested that the relationship of effects to hardness may vary among species, which presents challenges for the development of a species sensitivity distribution (SSD). First, data can only be combined across species with differing hardness-dependence if all species were tested at the same hardness, or if sufficient data exist to adjust all species for differences in hardness. Second, the resulting SSD can only be applied across the range of hardness for which all species in the SSD can be reliably hardness-adjusted. To address these challenges, we re-evaluated the evidence for variation in hardness dependence among species. We supplemented existing data by testing the sensitivity of *Ceriodaphnia dubia*, *Oncorhynchus mykiss*, *Hyalella azteca*, and *Pimephales promelas* to nitrate spiked into natural waters with hardness ranging from 160 to 485 mg/L as $CaCO_3$. Analysis of covariance supported a pooled hardness-correction slope to adjust existing data to a common hardness for derivation of an SSD, and to model how the SSD changes with changing hardness. We discuss the interpretation of this slope and the implications of different approaches to modelling hardness dependence in the context of an SSD.

WP174 A Proposed Revision to the draft Canadian Federal Environmental Water Quality Guideline for Triclosan

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Triclosan is an antibacterial and antifungal chemical applied to a variety of consumer products including soaps, detergents, moisturizers, and makeup. Environment and Climate Change Canada (ECCC) and Health Canada (HC) assessed potential risks of triclosan to Canadians and their environment under the *Canadian Environmental Protection Act* (CEPA, 1999),and published the final Assessment Report and Proposed Risk Management Approach in November 2016. The Assessment Report concluded that triclosan may enter the environment in a quantity or concentration or under conditions that may have an immediate or long-term harmful effect on the environment or its biological diversity. Subsequently, ECCC released a draft Federal Environmental Quality Guideline (FEQG) for triclosan in February 2017. The final guideline is to be used as an adjunct to the risk assessment and risk management of priority chemicals identified under the Chemicals Management Plan (CMP). As noted by the government, the use of FEQGs is voluntary unless prescribed in permits or other regulatory tools, and applies to the ambient environment. They are not effluent limits or "never-to-be-exceeded" values but may be used to derive effluent limits. The proposed FEQG value for triclosan derived by ECCC is an HC05 of 0.38 µg/L. We conducted an independent literature search, evaluated the reliability and relevance of the original studies selected by ECCC and additional studies identified through the literature search using ECCC's robust summary templates, and generated a revised triclosan dataset. The final data set was evaluated using the same statistical methodology used by ECCC. SSD Master v3.0 was used to generate the SSDs. The resulting recommended HC05 from the revised SSD is 0.85 µg/L. This presentation will carry you through the various steps and studies reviewed throughout this exercise, and present the revised species-sensitivity distribution (SSD) and HC05 guideline developed for consideration by ECCC.

WP175 Abiotic and Biotic Characteristics of Water Quality in the Strawberry River, AR and its Tributaries

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The Strawberry River is a sub-watershed of the White River Watershed Basin that flows from the Ozark foothills to merge with the Black River. The Strawberry River is listed by the ADEQ as an extraordinary resource and ecologically sensitive waterway, hosting several species of endangered freshwater mussels. However, sections of the river are listed as impaired for siltation and pathogens. The major cause of impairment in this watershed and its tributaries is the excessive turbidity from silt. Although the Strawberry River has multiple tributaries that play an important role in the chemical properties and species diversity of the main channel, their individual contribution is unknown. To improve sustainability in the Strawberry River, it is crucial to determine which of its tributaries are associated with greatest contaminants entering the river. The abiotic factors of eight tributaries were evaluated including total suspended solids, turbidity, pH, conductivity, temperature, DO, and total and dissolved nutrients. The p-value for the one-way ANOVA is less than 0.001 showing that the means for each of the tributaries differ. Tukey's post-hoc analysis indicate that TSS and turbidity increase in the downstream tributaries. The mean values for the most upstream and downstream tributaries are 3.1 and 11.09 mg/L, respectively. Nutrient analyses indicate comparable phosphorus values among sites but significantly greater values of nitrogen for two of the most downstream tributaries. This results suggest greater pollution on the areas where agricultural activity is higher. Freshwater mussel surveys performed up- and downstream of tributaries are used to compare potential impact to aquatic biota.

WP176 Analysis of the Potential Impact of Water Chemistry and Acute Toxicity on the Turtle Ecology at Medicine Lake in Plymouth, MN, USA

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Abiotic factors can perturb food webs and influence interactions amongst populations and communities. Freshwater systems contain nutrients and ions that have the potential to improve or diminish species niches. In this study, we analyzed aquatic habitat parameters that affect the use of an urban lake by a three species turtle community: *Chrysemys picta bellii, Chelydra serpentina,* and *Apalone spinifera*. Previous studies indicate chloride (Cl^-) may have a negative impact on aquatic species; according to the Canadian Environmental Protection Act of 1999, 5% of aquatic species will be affected by chronic exposure to Cl^- concentrations of 210 mg/L and 10% by 240 mg/L. A major source of Cl^- is road salt runoff. This issue warrants attention as the continued application of road salt in urban areas leads to increasing Cl^- in urban lake systems. We analyzed water chemistry in the summers of 2015 and 2016 in an urban lake in Plymouth, Minnesota, using YSI ProDSS Multi Parameter Water Quality Meters. We obtained pH, optical dissolved oxygen (ODO), oxidation-reduction potential (ORP), nitrate (NO_3^-), ammonium (NH_4^+), temperature (T), specific conductance and Cl^- at eight different habitats of the lake. These habitats were differentiated by hydrological factors and observed turtle locations. Preliminary data suggest no adverse correlations between pH, ODO, ORP, NO_3^-, or NH_4^+ concentrations and turtle locations. However, Cl^- ranged from 28 to 694 mg/L and these concentrations may have negative implications for aquatic food web dynamics. In spring 2017, shallow surface water samples were collected from the eight habitats and analyzed for acute aquatic toxicity (Delta Tox II Analyzer). Preliminary data show no evidence of acute toxicity, but deeper locations have not yet been analyzed. A positive correlation between Cl^- and water depth in four of the eight habitats was observed. Also, in deeper areas, lake water remained stratified into two distinct water layers with a recognizable difference in pH, T, specific conductance and Cl^-. A difference of at least 100 mg/L Cl^- was observed. These data indicate elevated Cl^- concentrations persist all year at deeper locations. Further analysis of the relationship between water quality parameters (Cl^-, ODO, T) and turtle ecology (movement, breeding, nesting, hibernation patterns) needs to be explored to best protect and conserve turtle populations in urban lake systems.

WP177 Aquatic Toxicity Testing for Aquatic Life Impact Assessments and Recent Scientific Advancements

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The focus of this presentation is to provide an overview of the use of aquatic toxicity testing for assessing possible impacts to aquatic life and how new scientific approaches are being researched. Toxicity testing of both ambient and effluent monitoring samples will be discussed as well as some of the Environmental Protection Agency's (EPA) toxicity testing recommendations. In the U.S., the Clean Water Act (CWA, 1972) established a national policy that the discharge of toxic pollutants in toxic amounts be prohibited. The use of aquatic toxicity testing, such as whole effluent toxicity (WET), under the National Pollutant Discharge Elimination System (NPDES) permits program, is an important component of EPA's integrated approach to water quality-based toxics control, along with aquatic life chemical-specific criteria and biological assessments. In this presentation, we will provide an overview of some of the key underlying scientific principles for generating representative and valid toxicity monitoring data which is used for water quality decisions affecting aquatic life protection. It will discuss the primary advantages of toxicity tests such as the evaluation of the integrated effects of all chemicals instantaneously in an aqueous sample and measuring the toxicity of chemicals for which EPA has not yet established numeric water quality criteria. It will discuss how toxicity testing can provide valuable insights into the ecological impacts to aquatic communities due to exposure to pollutants. Highlights of the important scientific principles and key technical considerations to support representative water quality toxicity monitoring will be covered as well as the various approaches to water quality toxicity monitoring. In addition, we will summarize current scientific advancements, research and the potential future direction of water quality toxicity monitoring such as additional test species and emerging scientific techniques (i.e., in vivo *to* in vitro testmethods). An overview of the ongoing international water quality toxicity monitoring discussions will be provided. The views expressed in this presentation are those of the author(s) and do not necessarily represent the views or policies of the Agency.

WP178 Bioenergetic Signatures of Stress in Caddisfly Larvae from Streams Along an Urban to Rural Gradient

M. Smith, Towson University / Environmental Science and Studies; A. East, Towson University / Environmental Sciences; K. McCreesh, Towson University; J. Moore, Towson University / Geosciences; C.J. Salice, Towson University / Biological Sciences / Environmental Science

Urbanization is an unavoidable feature of our modern environments that can have an array of impacts on imbedded or adjacent ecosystems. Included in these impacts are increased quantities of natural and anthropogenic material due to increased stormwater runoff; which is especially pronounced in urban environments. In the Mid-Atlantic, two potentially important inputs to streams mediated by humans are organic matter and salt ions. Once in aquatic systems, organic matter and salinity can lead to disturbances in organism physiology and, consequently, stream function. These quantifiable disturbances can be classified as stressors and may act in synergistic or antagonistic ways via alteration of bioenergetic processes. A challenge lies in characterizing and quantifying these disturbances. Organismal macromolecule ratios are hypothesized as one available metric to quantify the connection between stressor dynamics and disruption of bioenergetic processes. Five streams in Baltimore City and County, representing a rural to urban gradient (based on percent impervious surface of watershed), were sampled to determine whether patterns in macromolecules in stream invertebrates were indicative of stressors from urbanization. We predicted that organismal lipids would increase with urbanization. Carbohydrate, protein, and lipid content of benthic invertebrates (Trichoptera hydropsychidae) were extracted and quantified spectrophotometrically and compared against an urbanization ranking. Basic water chemistry data were also collected, including conductivity, total organic carbon and sediment lipid concentrations. Preliminary data showed differences among caddisflies from the five streams in macromolecule ratios and in total concentration and ratios between three lipid classes (neutral, glyco-, and phospholipids). However, we did not see a linear increase in lipids with urbanization, but instead saw a humped-shaped pattern. One possibility is that the increases in salinity associated with urbanization may counter influences of increases in available energy which would manifest as lower lipids in invertebrates from the most urbanized sites. Nonetheless, macronutrient patterns in invertebrates appears to provide a useful metric for exploring ecological effects of common anthropogenic stressors associated with urbanization.

WP179 Cellular Biomarker Responses of Freshwater Bivalves to Chlorpyrifos

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Chlorpyrifos is the number one active ingredient used in many insecticides in the United States, mainly used for corn, soybean, and alfalfa agriculture. Very high concentrations of chlorpyrifos have found throughout the Great Lakes, especially along the Maumee River. Chlorpyrifos can have negative effects on freshwater ecosystems and organisms. Bivalves are valuable bioindicator organisms that can be used to characterize ecosystem health and monitor the effectiveness of remediation programs. However many North American freshwater bivalves are threatened or endangered so studies with abundant invasive freshwater species such as *Corbicula fluminea* and Dreissena polymorpha were used to assess chlorpyrifos toxicity. One part of these investigations was in situ caging studies of *C. fluminea* and *D. polymorpha* conducted by the National Oceanic and Atmospheric Administration (NOAA) as part of the Mussel Watch program in the Maumee River Basin in Lake Eerie, in collaboration with the Environmental Protection Agency (EPA). Cellular biomarkers (including acetlycholinesterase and glutathione) of sublethal toxicity of clams and mussels from these field studies as well as laboratory exposures of *C. fluminea* to environmentally relevant concentrations of chlorpyrifos were conducted at UNCC. Adverse effects in both bivalve species related to pesticide exposures, especially acetylcholinesterase, were observed. These studies will facilitate insights regarding the impacts and extent of chlorpyrifos on environmental health.

WP180 Comparative studies of the effects of UV radiation and water accommodated fractions (WAFs) on the two congeneric copepods

J. Han, J. Lee, Sungkyunkwan University / Biological Science

To understand the effects of UV radiation and water accommodated fractions (WAFs) on the Antarctic (*Tigriopus kingsejongensis*)and the temperate (*T. japonicus*) copepods, we compared the deleterious effects of UV radiation and WAFs on mortality, reproduction, intracellular reactive oxygen species (ROS) levels, antioxidant enzymatic activity, and transcription regulation of defensome genes in two congeneric copepods. For UV exposure, the LD50-96h and no observed effect level (NOEL) at 96 h after UV radiation were determined as 23.16 kJ/m^2 and 12 kJ/m^2, respectively in *T. kingsejongensis*, whereas the LD50-96h and NOEL were 26.42 kJ/m^2 and 12 kJ/m^2, respectively in *T. japonicus*. In *T. kingsejongensis*, ROS levels at 12 kJ/m^2 UV exposure were slightly increased ($P<0.05$), while more significant increase were observed ($P<0.05$) in *T. japonicus*. In addition, transcript levels of antioxidant-related genes were mostly down-regulated in response to 12 kJ/m^2 UV radiation in *T. kingsejongensis*. *T. japonicus* heat shock protein (*hsps*) genes were mostly up-regulated but only small *hsps* (*hsp10* and *hsp20*) showed up-regulation in *T. kingsejongensis*. In case of WAF exposure, reproductive rates of two congeneric copepods were significantly reduced ($P < 0.05$). Furthermore, *T. kingsejongensis* showed elevated levels of ROS and higher antioxidant enzyme (glutathione peroxidase [GPx]) activity than *T. japonicus* in response to WAFs. Also, *CYP* genes from congeneric copepods were identified and annotated to better understand molecular detoxification mechanisms in response to WAFs. We observed significant up-regulation ($P < 0.05$) of *Tk-CYP3024A3* and *Tj-CYP3024A2* in response to WAFs, suggesting that *CYP* genes may contribute to the detoxification mechanism in response to WAF. Taken together, these findings suggest that UV radiation and WAFs may induce oxidative stress, leading to physical dysfunction in two congeneric copepods. Overall, this study provides a better understanding of how UV radiation and WAFs affects in vivo endpoints and the relevant molecular response in two different copepod species from contrasting environments.

WP181 Comparison of methods for bioassessment of urban streams to assess water quality changes associated with green infrastructure and restoration projects

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Monitoring and assessing effectiveness of Green Infrastructure (GI) and remediation/ restoration in urban environments with flashy hydrology presents a number of challenges. Though ideally GI, remediation, or restoration would be done at a watershed scale, population and land use density in urban catchments leads to fragmented and often uncoordinated investment in and implementation of these efforts. Hydrology and water quality of streams is often impacted by flashy flows, Combined Sewer Overflows (CSOs), industrial discharges, septic systems, wastewater treatment plant effluents, irrigation, roadway runoff and a high percentage of impervious surfaces in urban drainage basins. Efforts to characterize impact from sporadic placements of GI or restoration is therefore complicated by the density and variety of stressors to urban streams. Bioassessment can provide useful metrics to determine remediation or restoration effectiveness for urban streams. It can be used to demonstrate improvement in ecological condition and can integrate effects from a wide range of stressors. Bioassessment of water quality by retrieval and analysis of macroinvertebrate communities is well established but entails a unique set of challenges with respect to urban streams for assessment of associated GI and restoration projects. In order to maximize the ability to detect differences in communities before and after projects or in different reaches, a macroinvertebrate sampling method which is the most quantitative and retrieves a high proportion of the taxa residing in a stream reach is desired. In conjunction with GI and restoration projects planned on Cincinnati's Mill Creek and one of its tributaries, Congress Run, we are comparing macroinvertebrate sampling methods for their usefulness for bioassessment of these flashy urban streams. We assessed 3 methods: 2 artificial-substrate and one fixed-area method. Gravel trays, modified 'low profile' Hester-Dendy multi-plate samplers (to deal with the lack of sufficient depth in headwater streams), and a 'bucket' method, were compared in terms of usefulness in flashy urban streams, number of taxa retrieved, and by variability of the results. Samplings in 2016 and 2017 showed that the artificial substrate samplers performed well with respect to variability, but the sampler with the most advantages was the fixed-area "bucket" sampler, which performed well with respect to number of taxa retrieved and usefulness.

WP182 Considerations for Assessing the Protectiveness of EPA Aquatic Life Criteria to Threatened and Endangered Species

J. Justice, USEPA; C. Bergeron, K. Gallagher, USEPA / Office of Water

The U.S. Environmental Protection Agency (EPA) consults with the U.S. Fish and Wildlife Service (FWS) and the National Marine Fisheries Service (NMFS; collectively, the Services) under Section 7 of the Endangered Species Act (ESA) when approving certain changes to state and tribal water quality standards, and when proposing or promulgating certain Federal water quality standards under Section 303(c) of the Clean Water Act (CWA). To support Section 7 ESA consultation, EPA typically assesses whether the presence of a particular pollutant at criterion magnitude, duration, and exceedance frequency is likely to adversely affect any pertinent listed species or their critical habitat. Understanding listed species sensitivity to pollutants, however, is often confounded by the relatively limited species-level toxicity data available to support listed species effects assessments. EPA Health and Ecological Criteria Division (HECD) is, therefore, exploring the use of taxonomic surrogate toxicity data and Interspecies Correlation Estimates (ICE) to understand listed species sensitivity to pollutants at criteria magnitudes when species-level toxicity data are lacking. Species-level or appropriate surrogate concentration-response data can then be evaluated to identify listed species low level effect concentrations (e.g., LC_{05}, EC_{05}). Additionally, HECD is further assessing the utility of time-dependent toxicity data to account for protection provided by the duration component of most criteria. For example, acute and chronic toxicity values used to support ESA effects assessments are typically based on continuous laboratory exposure of 96 hours and 28 – 60 days, respectively, which are significantly longer than most acute and chronic criteria durations of 1 hour and 4 days, respectively. Considering listed species effect concentrations assessed over multiple exposure durations may provide a robust understanding of listed species sensitivity to pollutants at corresponding criteria magnitudes and durations. Where listed species data are limited, taxonomic surrogate or estimated data, in combination with time-dependent toxicity data, may provide insight into listed species sensitivity to pollutants under environmentally-relevant exposure scenarios.

WP183 Cross-taxa distinctions in aquatic toxicity between representative species for risk assessment

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Standard ecological risk assessment practices often rely on larval and juvenile fish toxicity data as representative of the amphibian aquatic stage. Although empirical evidence suggests fish early life stage toxicity values frequently are sufficiently sensitive to protect larval amphibians, the process of metamorphosis relies on endocrine cues that affect development and morphological restructuring. Altered developmental effects can lead to detrimental impacts at the population level that are not captured in standard early life stage fish tests. This study compares developmental endpoints for zebrafish (*Danio rerio*) and the African clawed frog (*Xenopus laevis*), two standard test species, exposed to the herbicide trifluralin for 30 and 70 days, respectively. Zebrafish were more sensitive in acute toxicity and demonstrated a reduction in growth measurements with increasing trifluralin exposure. Growth measurements in *X. laevis* at metamorphosis were not correlated with exposure; however, time to metamorphosis was delayed relative to trifluralin concentration. Nonmonotonic growth effects and elevated thyroid hormone levels suggest compensatory molecular mechanisms are responsible for complex endocrine responses to trifluralin in amphibians. Such responses are not completely represented by early life stage fish tests. Additional comparison of gene expression will contribute to identification of key molecular initiating events representative of complex alterations in biochemical pathways. Interpretation of developmental effects in the context of altered vital rates will improve assessment of sublethal impacts on amphibian species.

WP184 Dietary carbon and lipid content influences population dynamics of Daphnia magna

T.J. Woo, Towson University / Environmental Science; C.J. Salice, Towson University / Biological Sciences/ Environmental Science

Standard laboratory protocols for testing chemical toxicity using *Daphnia magna* call for *ad libitum* feeding of a high quality alga. In natural environments, *Daphnia* likely feed on diverse communities of phytoplankton that differ in quantity and quality. Hence, standard laboratory feeding practices may conceal impacts of diet quantity and quality on *D. magna* life history traits and population dynamics that are ecotoxicologically important. To provide insight into these impacts, a laboratory study was conducted in which *D. magna* populations were maintained for 42 days using two algae, *Raphidocelis subcapitata (RAP)* and *Nannochloropsis sp. (NAN)*, and two feeding levels. *NAN* was expected to be the superior quality algae, having higher carbon and lipid content per cell. Diet treatments were thus normalized for mg C L^{-1} day^{-1} and cells L^{-1} day^{-1}; *RAPlow* and *NANlow* were normalized to 0.19 mg C L^{-1} day^{-1}, *NANhigh* was normalized to the cell density in the *RAPlow* treatment, and *RAPhigh* was normalized to the mg C in the *NANhigh* treatment. Populations were counted three times a week, and organismal length was assessed weekly. Individuals from sacrifice populations were analyzed for carbon and lipid content on day 21. *RAPlow* and *NANlow* had similar population trajectories, while *RAPhigh* populations reached the highest peak sizes and had the highest rate of decline. Interestingly, *NANhigh* populations peaked very low before crashing to extinction. At day 21, *Daphnia* from *RAPlow* and *NANlow* treatments had similar organismal length distributions with two age classes of neonates and adults, while *RAPhigh* had more continuous length distributions. At day 21, adults in *NANlow* and *RAPhigh* treatments had similar carbon and lipid content, both higher than in *RAPlow*. Neonates showed the opposite pattern, where *RAPlow* neonates had higher carbon and lipid content than both *NANlow* and *RAPhigh* neonates. The results of this study suggest that dietary carbon and lipid content influences *D. magna* population dynamics, and alters patterns of maternal lipid and carbon investment into offspring. Percent carbon and percent lipids are common metrics for organismal health and energy; therefore the results of this study have broad implications for toxicity tests where these measures are not required. Furthermore, these results point to the importance of considering the resource environment during efforts to protect sensitive populations.

WP185 Disruption of lipid metabolism in developing zebrafish with exposure to perfluoroalkylated substances (PFASs)

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Perfluoroalkylated substances (PFASs) are persistent organic pollutants that have been reported in water supplies, as well as human serum samples. Exposure is associated with a number of hepatic effects. The current study examines effects on lipid metabolism in the developing zebrafish. The PFAS are categorized into long chain (7 or more carbon atoms in their backbone) and short chain (6 or fewer carbon atoms) compounds. Three long chain PFASs, perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), and perfluorononanoic acid (PFNA); and 2 short chain PFASs, perfluorohexanoic acid (PFHxA) and perfluorohexane sulfonate (PFHxS) were examined in this study. Embryos were exposed to static non-renewable PFAS treatments (0.02- 2 µM) from 3 hours post fertilization (hpf) to 120 hpf. At 120 hpf, larvae were transferred to treatment-free water until 2 weeks post fertilization at which point morphometric and hepatic Oil Red O lipid staining measurements were recorded. Exposure to 2 µM long chain PFASs significantly increased hepatic lipid staining and caused a significant reduction in total body length. Exposure to 2 µM short chain PFASs did not result in any significant changes in these parameters. In addition, there were no significant changes in liver area of the larvae. Based on preliminary findings we hypothesize that the long chain PFASs will react differently than the short chain PFASs as it relates to lipid metabolism. Therefore, expression of genes related to lipid metabolism, such as acyl-CoA synthetase (*acs1*), apolipoprotein (*apoa1, apoc2*), lipase (*lipg*), microsomal triglyceride transfer protein (*mtp*), insulin receptor (*insr*), and fatty acid transporter (*slc27a*) will be examined. Alteration in regulation of these genes has been demonstrated in rodent models exposed to PFASs and may or may not serve as targets in the zebrafish. These studies may help to elucidate the mechanisms of hepatic steatosis and other alterations in lipid metabolism reported in the zebrafish.

WP186 Effect of atrazine on the sex ratio of the Neotropical fish, Odontesthes bonariensis, exposed during the thermolabile window of sex determination

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Atrazine is an herbicide heavily used worldwide. Due to its physicochemical properties and environmental behavior, it is frequently found in the aquatic ecosystems. In addition, a lot of evidence exist on the adverse effects of atrazine on aquatic vertebrates, particularly acting as an endocrine disrupting compound (EDCs), modulating aromatase activity. The inland "Pejerrey" (*O. bonariensis*), is characteristic fish of the southern sector of the Río de la Plata Basin. In addition, Pejerrey presents thermolabile-sex determination (TSD) that happens between the 2nd and 6th week after hatching and therefore it could be particularly sensitive to EDCs. All females or males are expected at 19°C or 28°C, respectively, while 50% female and male are expected at 25°C. In the present study we evaluate for the first time, the effects of atrazina on the sex determination of Pejerrey during the thermolabile window. Twenty-five fish were exposed in 60L stainless steel tanks to 0, 0,5, 5 and 50 µg/L of atrazine (a.i.) from the 2nd to 6th week after hatching, by duplicated. In addition, a group was exposed to 0,025 µg/L of EE_2 as positive control. Ten fish per treatment were sampled at week 6 for molecular biology and 15 fish were later sampled at week 12 for histology. Gonadal histology showed that in the control group, 60% were males and 40% females while in the positive control 100% of fish were females. In fish exposed to the highest concentration of atrazine, 73% females, 18% undetermined and 9% males were obtained. This first obtained results suggest that the herbicide was able to skew the sex ratio, feminizing Pejerrey during the thermolabile window.

WP187 Effects of 4-methylcyclohexane methanol (MCHM) on the predator-prey interaction between Danio rerio and Daphnia magna

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Chemical contamination alters organism-level traits, such as activity and feeding, that can ultimately affect aquatic trophic interactions. Despite the importance of predator-prey relationships in aquatic communities, chemical toxicity is often tested on single species prior to use. For example, 4-methylcyclohexane methanol (MCHM) used in industrial coal-cleaning enters the environment regularly from low-level contamination during disposal and occasionally in high concentrations following accidental spills, but its effects on fish-zooplankton interactions remain unknown. Zebrafish (*Danio rerio*) and *Daphnia magna* are ideal species for studying chemical effects on predator-prey interactions because the traits and behaviors of both organisms are well studied. Although acute exposure to MCHM increased *D. magna* immobility and reduced larval zebrafish activity, the potential for MCHM to persist in the environment suggests exposure should also be tested over chronic time frames. We hypothesized that chronic exposure to MCHM affects the behavior of zebrafish and *D. magna* individually, and their predator-prey relationship. To test this hypothesis, we first quantified the effects of environmentally relevant concentrations of MCHM on zebrafish and *D. magna* activity over one week. Zebrafish swimming distance and velocity and *D. magna* looping behavior and immobility were measured using behavioral analysis software 1, 3, and 7 days post-exposure to one of four MCHM concentrations (0.5, 1, 3, or 5 ppm) or a no addition control. Results suggest MCHM decreased both zebrafish activity and *D. magna* looping behavior, and increased *D. magna* immobility over the course of 7 days. Therefore, MCHM could change the predator-prey relationship by either reducing zebrafish feeding activity, or increasing zebrafish feeding by preventing *D. magna* escape behavior. This study highlights the importance of multi-species tests to better constrain the effects of toxins in aquatic environments.

WP188 Effects of atrazine and a simulated herbicide mixture on two North American sentinel species, the northern leopard frog and the fathead minnow

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The two objectives of this study were: 1) to determine if fathead minnows (Pimephales promelas) and northern leopard frogs (Rana pipiens) exposed to atrazine experience alterations in endocrine-responsive gene expression, and 2) to determine whether a simulated environmentally-relevant herbicide mixture causes changes in gene expression that are consistent with those elicited by atrazine alone. Treatment groups for both fish and frogs consisted of a solvent control (0.0025% acetone), three atrazine doses (0.5 µg/L, 5.0 µg/L, and 50.0 µg/L), and the reconstituted river mix. The reconstituted river mix contained: atrazine (5.0 µg/L) acetochlor (3.6 µg/L), desisopropylatrazine (0.8 µg/L), metolachlor (0.5 µg/L), deethylatrazine, 0.4 (µg/L). Animals were assessed for changes in body gonadosomatic index (GSI), hepatosomatic index (HSI) and estrogen responsive mRNA expression of vitellogenin (Vtg) and estrogen receptor α (ERα) by RT-qPCR analysis. No changes were detected in the GSI and HSI of fish and frogs. Increased ERα expression was detected in male frogs at the 50.0 µg/L atrazine relative to solvent controls, while increased Vtg concentration was observed in female fish at 5.0 µg/L of atrazine. No changes in detected in the environmentally relevant mixture of herbicides in fish or frogs. These observations demonstrate that atrazine alone is capable of eliciting changes in endocrine biomarkers whereas its biological effects can be masked when minnows or frogs are exposed to herbicide mixtures.

WP189 Effects of Nickel on cell cycle progression, growth and antioxidant enzymes of green algae Chlamydomonas reinhardtii

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Freshwater ecosystems received industrial-domestic sewage discharges, chemical compounds from anthropogenic activities. Metals released in the environment have increased over the last decades causing environmental problems worldwide. The study of the effects of metal on aquatic organism is relevant, they are not biodegradable and persistence. Microalgae have an important role in aquatic system as they are a key component of food chains. It is crucial to has early assessment tools to evaluate effects at the cellular level, it can lead to disturbance in structure and productivity of the algae community. In the present study effects of Ni was evaluated on cell cycle progression, growth, antioxidant enzymes kinetic of *C. reinhardtii*. Synchronized cultures of this multiple fission dividing algae were used for the study. Aliquot from growing cultures were taken hourly during 36 hours. The attainment of commitment points (CP) was evaluated by transferring hourly aliquot into aerated tubes at30 °C in the dark. In hourly samples analysis of cellular division, nuclear division (DAPI stain) changes in cell size, protein, antioxidant enzymes activities of catalase, guaiacol peroxidase, ascorbate peroxidase, glutathion reductase were performed. The proportion of mother cells and daughter cells were assessed at the end of the cell cycle. Ecotoxicity of metal was assessed by algal growth inhibition test, estimating toxicity endpoints, growth rates and concentration of chlorophyll a, chlorophyll b and carotenoids at the end of 96 hs of exposition. Ni provoked a block of cell cycle at the highest concentration tested. At lower concentrations, cell cycle progression was observed with different pattern of attained CP, depending the exposure concentration. Antioxidant enzyme activities were inhibited at concentration above 0,05 - 1 mg/L. The effects of metal on pigments was less evident than the effects on growth rates, indicating a lower sensitivity of these parameters. Ni provoked severe damage on algal cell growth, cell cycle progression, photosynthetic pigments as well as modification of antioxidant enzymes activities. An integrated analysis is done discussing the consequences on population performance in natural environment affected by heavy metal discharged from different anthropogenic sources. The importance of developing integrated studies at different cellular level is pointed out in order to avoid/predict the impact of metal discharge on aquatic environments.

WP190 Effects of Sublethal Chlorpyrifos Exposure on the American Lobster (Homarus americanus)

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The American lobster (*Homarus americanus*) is an economically important species for Atlantic regions of Canada and the United States. Landings from Canada and the US for 2015 combined were over 150,000 metric tonnes and with a value of over $621 million USD. Larval lobster life-stages exist in both the pelagic and benthic zones and are therefore vulnerable to agricultural runoff of pesticides. The organophosphate chlorpyrifos, a pesticide that targets arthropodic pest insects, has been shown to affect the survival of some larval decapod crustaceans, yet no data exists on the impacts to *H. americanus*. Using 48-hour acute exposures, the median lethal concentration (48 h) of chlorpyrifos was established to be 1.56 ± 0.50 µg/L for stage IV American lobster. During sublethal exposures, growth parameters were found to be significantly affected at the tested chlorpyrifos concentration of 0.82 µg/L. Using general linear model analysis ($\alpha = 0.05$), it was determined that larvae exposed to this treatment displayed significant increase of intermoult period and significant decrease of both specific growth rate and moult increment when compared to the control treatment (< 0.03 µg/L chlorpyrifos). RNA sequencing was performed using Illumina Hiseq 4000 PE100 and subsequent confirmation of expression of genes of interest was performed via RT-qPCR. In the current study, gene expression was used to determine pathways being affected by sublethal chlorpyrifos exposures. Unique patterns of gene induction may serve as a potential diagnostic tool to further examine the impacts of pesticides on lobster.

WP191 Effects of water-borne benzo[a]pyrene on early-life stages of the fathead minnow (Pimephales promelas)

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Polycyclic aromatic hydrocarbons (PAHs) are a class of ubiquitously distributed environmental pollutants that mainly originate from petrogenic and pyrogenic sources such as combustion of fossil fuels and other organic material. Various PAHs, including benzo[*a*]pyrene (BaP), have been demonstrated to cause a wide range of effects in exposed wildlife, including alterations of immune responses, impaired development and reproduction, as well as mutagenesis and carcinogenesis. Most studies to date, however, have used comparably high exposure concentrations, dietary routes of exposure or intraperitoneal injection to administer BaP, and knowledge of low-dose effects at concentrations around water solubility (approx. 4 µg/L) is generally limited. This route of exposure, however, must be considered highly relevant in light of the distribution of PAHs even into remote aquatic systems. To bridge this knowledge gap, early-life stages of the fathead minnow (*Pimephales promelas*) will be exposed to waterborne BaP as a model compound to characterize toxicity pathways that drive the sensitivity of early life-stage fish to PAHs. Molecular responses at the whole transcriptome, proteome and metabolome level will be investigated at the swim-up stage, and quantitatively correlated with effects on apical (growth, survival, development), histopathological, and biochemical endpoints 28 d post-hatch. The data generated within this experiment will help to better understand the relevance of aqueous exposure to BaP specifically, and PAHs in general, and provide important insights into the relevance of molecular responses in early-life stages as early-warning biomarkers for apical outcomes in juvenile and/or adult fish. This study is part of the EcoToxChip project (@ecotoxchip).

WP192 Environmentally-relevant exposure to tributyltin induces premature hatching and reduces locomotor activity in zebrafish (Danio rerio)

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Tributyltin (TBT) is an organotin compound that is the active ingredient of many biocides and antifouling agents. In addition to its well established role as a chemical with an anti-androgenic mode of action, evidence also suggests that exposure to tributyltin (TBT) adversely affects the nervous system and behavior. However, the mechanisms underlying TBT-induced behavior dysfunction remains unclear. In this study, zebrafish (*Danio rerio*) embryos were exposed to environmentally relevant concentrations of TBT (0.01, 0.1, 1 nM) for 6 days. Hatching time were significantly decreased for fish exposed to 1 nM TBT and after 96-h exposure, total swimming distance, velocity, and activity of zebrafish larvae were reduced compared to controls. Following 24 and 48-h exposure to TBT, there were no differences in endpoints related to mitochondrial bioenergetics in the embryos that included basal respiration, mitochondrial respiration, and maximal respiration, suggesting that TBT did not negatively affect bioenergetics during development at environmentally relevant concentrations. Moreover, the expression levels of genes related to muscle function (*myf6*, *myoD*, and *myoG*) and dopamine signaling (*th*, *dat*, *dopamine receptors)* were not affected by TBT in the larvae, suggesting that the behavioral differences observed did not involve modulation of the dopamine system at the transcript level. Our data show that TBT can induce premature hatching and locomotion dysfunction in larvae, but changes in these higher level endpoints appear not to be related to impaired mitochondrial bioenergetics nor to the expression levels of transcripts involved in muscle function nor dopamine signaling.

WP193 Estimating species sensitivity distributions for nitrite from limited data

E. Costa, Golder Associates, Ltd.; A. deBruyn, Golder Associates / Environmental Science

Nitrite disrupts multiple physiological functions in fish, including ion regulation, respiration, and cardiovascular, endocrine, and excretory processes. Largely as a result of its potential to affect aquaculture, the acute toxicity of nitrite to fish, and the toxicity-modifying effect of chloride, are well studied. However, relatively few acute toxicity data exist for other taxa, and very few chronic toxicity data exist for any species. We explored the use of acute-to-chronic ratios (ACRs) and other extrapolation methods to estimate species sensitivity distributions (SSDs) from limited data and to characterize the effect of chloride on those SSDs. We also discuss the ACRs estimated for nitrite in the context of mechanisms of toxicity.

WP194 Evaluating the Impacts of Road Salt Runoff on Pond Snails

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Deicing salts are commonly applied during the winter months to prevent roads and sidewalks from freezing and increase traction. Once dissolved in run-off, dissociated chloride ions can rapidly enter aquatic ecosystems, and increases in salinity caused by road salts threaten entire aquatic ecosystems. Macroinvertebrates are crucial organisms at many trophic levels within aquatic ecosystems, and these organisms are sensitive to elevated salinity. By impairing macroinvertebrate communities, increases in salinity caused by road salts threaten entire aquatic ecosystems. The purpose of this study was to assess the effects of deicing salt on the salinity of five freshwater ecosystems on the campus of Kent State University. Additionally, chronic toxicity tests evaluated the growth and fecundity of pond snails (*Lymnaea stagnalis*) at increasing levels of salinity. Water samples from five locations were collected following a snow event and were measured for conductivity and ionic content. Individual snails (1–3 days old) were introduced to these water samples, and growth and mortality were monitored over a period of four weeks. Additional bioassays were conducted to assess snail fecundity when exposed to reconstituted stormwater of varying salinities. Preliminary results showed that pond snails exposed to highly saline water grew significantly larger than snails exposed to lower levels of salinity. However, newly hatched snail mortality occurred more frequently in more saline water samples, potentially indicating that young snails are less tolerant to saline freshwater than adults.

WP195 Impacts of major freshwater ions on the acute toxicity of silver nanoparticles to Daphnia magna

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Silver nanoparticles (AgNPs) are increasing in presence in commercial and medical products due to their bactericidal properties. Aqueous silver (Ag^+) toxicity to freshwater organisms has been well studied using the Biotic Ligand Model (BLM), a model that predicts metal toxicity at reactive biological surfaces of freshwater organisms in relation to their surrounding water chemistry. Although dissolved Ag^+ from the AgNP surface is still being debated as the toxic mechanism of action of AgNPs, toxicity via ionic dissolution from AgNPs is not accurately predicted by the BLM, which suggests the existence of other or additional chemical or biological reactions. The purpose of this research is to observe the effects of the major freshwater ions Ca^{2+}, Na^{+}, Cl^{-}, and NO_3^{-} on the toxicity of AgNPs to the freshwater daphnid, *Daphnia magna*. These ions can change the kinetic behavior of the AgNPs by increasing freshwater concentrations of Ag^{+} via ionic dissolution of the particles or, contrarily, reducing the reactive surface area of the particles as they aggregate and sediment out of a water column. This study uses 8 acute toxicity tests performed in ASTM moderately hard synthetic freshwater that have varying additions of Ca^{2+}, Na^{+}, Cl^{-}, and NO_3^{-}. The BLM predicts that all experimental synthetic freshwaters will reduce Ag^{+} toxicity in comparison to ASTM moderately hard water without additions, driven in chief by the concentration of total ions in solution. Nanoparticle kinetic theory also predicts a reduction of dissolved Ag+ concentration driven by a combination of major ion concentration and valency, theoretically reducing Ag+ toxicity from AgNPs. Preliminary tests have shown that both of these mechanisms influence toxicity with increases of LC50s much higher than what the BLM predicts for each experimental synthetic freshwater. Multivariate analysis is used to compare AgNP toxicity to the BLM predictions for Ag^{+} toxicity as well as different particle behaviors, measured using single particle ICP-MS and UV-Vis spectrometry. This research aims to further inform the debate about the toxic mechanism of action for AgNPs, as well as understand the impact of specific major ions in freshwater ecosystems on the environmental toxicity of AgNPs.

WP196 In vitro Genotoxicity of Surface Water and Wastewater from Lake Ontario in freshwater mussels

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A variety of chemicals can induce damage to the DNA of animals, which, if not repaired, can lead to a cascade of biological effects at any level of biological organization. First developed in the 1980s, the single cell gel electrophoresis assay ? or Comet assay - is used to visualize and quantify cellular DNA damage. In recent years, a number of studies have used this approach to evaluate DNA damage from exposure to specific chemicals (e.g., PAHs), as well as from complex mixtures of contaminants. Several regions of the Great Lakes have suffered from considerable habitat degradation due to the influence of intensive industrial, agricultural and urban development. In collaboration with the Ontario Ministry of the Environment and Climate Change, we developed an in vitro Comet assay to assess DNA damage in the hemocytes of the freshwater mussel Elliptio dilatata exposed to surface water and wastewater influent and effluent extracts from three sites in Lake Ontario: Hamilton Harbour, Toronto Harbour and Humber Bay. These extracts induced modest DNA damage after a 4 h in vitro exposure. Surface water extracts from Hamilton Harbour slightly induced DNA damage compared to the control, particularly surface water from the west end of the Harbour (12.5 % and 25% extracts only), and an Index Station located in the middle of the Harbour (100% extract). These results were somewhat expected, considering that sediments in the harbour are highly contaminated with genotoxic compounds. Significantly increased DNA damage was observed in mussel hemocytes exposed to three of the Toronto Harbour wastewater influent samples (12.5%, 25% and 50% extract) collected in June 2015, however these results were not consistent with the same samples collected in October 2015. The Humber Bay extracts did not appear to be particularly genotoxic, with the exception of the 100% extract for the Humber River surface water plume collected in October 2015. In the case of the Toronto Harbour data, the results of the present study indicate that wastewater treatment may successfully reduce the genotoxicity of the influent. Multivariate analyses are underway to investigate potential relationships between the induction of DNA damage and chemical (i.e. metals, PCBs, pharmaceuticals, antibiotics) concentrations in the samples examined.

WP197 In vivo quantification of cytochrome P450 activity in freshwater invertebrates

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Mixed function oxygenases (MFOs) comprise an organism's first line of defense against xenobiotics. MFOs exhibit broad substrate specificities, which allow them to alter/detoxify a wide variety of endogenous and exogenous compounds. Consequently, MFO activity plays an important role in dictating sensitivity to xenobiotics. The current study aims to validate an in vivo assay that quantifies metabolic activity in response to multiple MFO substrates simultaneously in three freshwater invertebrate species: *Daphnia magna*, *Physa acuta*, and *Chironomus dilutus*. Organisms were exposed to 6 µM concentrations of three substrates—4-nitroanisole (4-NA), 7-ethoxycoumarin (7-EC), 1-naphthyl acetate (1-NpA)—for six hours. Subsequently, the metabolites of these substrates were extracted from the water using a three-step organic extraction. To improve the quantification of these compounds, the extracted samples were incubated with a derivatization agent (BSTFA; [N, O-Bis[trimethylsilyl] trifluoroacetamide]) at 60?C for one hour, after which they were analyzed via gas chromatography. This assay permits a comprehensive evaluation of xenobiotic metabolism across a range of invertebrate taxa.

WP198 Multiple biomarker approach to assess estuarine pollution: A New Zealand estuary case study

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Estuaries are the most valuable and dynamic natural habitats in the coastal zone. However, they are one of the most threatened marine ecosystems and are impacted by numerous anthropogenic activates and exposed to continuous influxes of a wide variety of contaminants from industrial waste, agricultural runoff, recreational boating and municipal wastewater discharges. Continuous monitoring of the environmental health of estuaries is critical to protect, conserve and manage those vital ecosystems. Biological monitoring is a useful tool as it is more ecologically relevant than chemical monitoring. The estuarine gastropod, *Amphibola crenata* was investigated as a suitable bioindicator to evaluate trace metal pollution in New Zealand estuaries. Snail samples were collected from different estuarine sites in New Zealand with varying contaminant concentrations. The overall goal of this study was to define a set of biological monitoring tools for estuaries for use in environmental assessment and monitoring. The results of integrated assessments of multiple biomarkers including trace metal content (Cu, Zn, As, Ni, Cd and Pb) of individual tissues, physiological (oxygen consumption and ammonia excretion), biochemical (haemolymph glucose, tissue catalase and protein) and reproductive biomarkers will be presented. Trace metal bioaccumulation in body tissues varied with tissue type and availability of metals in the environment. Physiological and biochemical biomarkers also showed site-specific variations based on the bioavailability of contaminants while the reproductive performances of *A. crenata* were highly variable under different environmental stressors. Overall the study shown that environmental changes are reflected in the biomarkers of *A. crenata*.

WP199 Pesticide Impacts from Louisiana Rice Fields on the Health and Survival of Red Swamp Crayfish

E.N. Vebrosky, Louisiana State University / Environmental Sciences; K. Armbrust, Louisiana State University / Environmental Sciences School of the Coast and Environment; W. Xu, Louisiana State University AgCenter / Renewable Natural Resources

The health and quality of both rice and crayfish production are immensely important to the culture and economy in the state of Louisiana. Ensuring the highest yield of rice requires the use of pesticides and flooding to deter pests from destroying crops. Flooding of the fields provides very suitable habitat for crayfish and crayfish farms. While there are many species of crayfish that call Louisiana home, the "red swamp crayfish" (*P. clarkii*) is the most abundantly farmed and harvested for human consumption.

Therefore, understanding the impacts that rice field pesticides have on the health and overall survival of these crayfish is important. Aside from pesticide exposure, byproducts such as photodegradation products of these pesticides pose just as big of a risk to the crayfish as the parent compounds. Juvenile crayfish are exposed to various concentrations of pesticides and also exposed to artificial sunlight to determine toxic and phototoxic effects the chemicals may have on the organisms. Pesticides, such as dicloran (Botran™; 2,6-dichloro-4-nitroaniline), have shown to have phototoxic implications at concentrations as high as 1 ppm in the presence of sunlight exposure for 24 hours (>50% mortality). Morphological changes in tissues of survived crayfish from the toxicant exposure were analyzed histologically and compared to the normal healthy individuals. Molecular analysis of the survivors' host anti-stress responses were also evaluated with quantitative PCR. This model can be applied to other pesticides that are more heavily used within Louisiana on rice fields to observe any lethal or sub-lethal impacts they may have on the organisms.

WP200 Pharmaceutical, PAH and PCB hepatic in vitro biotransformation in two species of Gulf of Mexico sharks

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The Galveston Bay watershed comprises a catchment area for major industrial, agricultural and municipal pollutant discharges. As a result, Galveston Bay and its surrounding areas are key focus areas for environmental health monitoring and remediation efforts. For example, routine surveys typically quantify elevated levels of PAHs, PCBs, dioxins and related compounds in resident biota, water and sediment around Galveston Bay and the Houston Ship Channel. Therefore given the susceptibility of resident biota to exposure, it is not fully known the extent to which exposed organism's biotransform or metabolize various classes of pollutants. In this poster, we present the intrinsic hepatic biotransformation capabilities of two species of Gulf of Mexico sharks, namely bonnethead (*Sphyrna tiburo*) and blacktip (*Carcharhinus limbatus*) sharks. These species were chosen as their habitat ranges include coastal and estuarine regions vulnerable to pollution. Our studies show inter-species differences in hepatic phase I (cyp1a1) activity as measured using the EROD assay (significantly elevated in bonnethead sharks). Preliminary studies using hepatic S9 fractions show selected pharmaceutical compounds to be readily metabolized relative to PAHs and PCBs. The results presented allow assessment of varying species capabilities to metabolize commonly found environmental pollutants, potentially influencing pollutant bioconcentration/bioaccumulation potential.

WP201 Phototoxic Effects of Pesticides Including Dicloran to Juvenile Red Swamp Crayfish and Eastern Oysters

E.N. Vebrosky, Louisiana State University / Environmental Sciences; K. Armbrust, Louisiana State University / Environmental Sciences / School of the Coast and Environment; W. Xu, Louisiana State University AgCenter / Renewable Natural Resources; L. Basirico, Louisiana State University

Louisiana's culture and economy rely heavily on the health of many organisms consumed inside and outside the state borders, including crayfish and oysters. The Red Swamp Crayfish (*P. clarkii*) is one of many species of crayfish found within Louisiana's waters, but it is the most heavily harvested for consumption. Eastern oysters (*C. virginica*) are another staple in many Cajun and Creole cuisines. Both organisms are at risk for a wide array of chemical and toxicological impacts due to their habitat locations. Runoff from agriculture fields can enter the surface waters of the neighboring systems they call home and can have adverse effects on the overall quality, health, and survival of crayfish and oysters. Chemical runoff into surface waters is highly susceptible to photolysis, which has the potential to increase toxic impacts through photo-induced toxicity; these impacts can be more harmful to the health of organisms than the parent-compound alone. Implications for toxic and phototoxic impacts were observed by mortality of crayfish after exposure to dicloran (2,6-dichloro-4-nitroaniline) and artificial sunlight over a 24-hour period at concentrations reaching 1 ppm maximum (>50% mortality). The influence of dicloran on oysters was evaluated using primarily cultured oyster heart myocytes, which was previously used as an indicator of environmental implications. Alterations of cell proliferation, apoptosis, and myogenic contraction under stresses of different concentrations of dicloran were measured and used as parameters for the evaluation of dicloran toxicity.

WP202 Predicting In Situ Responses of Taste- and Odor-Producing Algae in a Southeastern US Reservoir to a Sodium Carbonate Peroxyhydrate Algaecide

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Laboratory experiments can be used to develop models of organism responses to hydrogen peroxide (H_2O_2) exposures. For sodium carbonate peroxyhydrate (SCP), a granular form of H_2O_2 and a relatively unstudied material in aquatic environments, preliminary laboratory studies can be used to develop exposure-response models to predict and interpret in situ exposure-response data. An application of Phycomycin® SCP, an SCP algaecide, was used in a drinking water reservoir (Hartwell Lake, Anderson, SC) to control a benthic algal assemblage putatively producing the taste- and odor-compounds 2-methylisoboreol (MIB) and geosmin. This episodic SCP exposure provided an opportunity to test hypotheses regarding the potential convergence of a laboratory model with in situ exposures and responses. Objectives of this study were to 1) measure responses of a benthic algal assemblage from Hartwell Lake to 7-d laboratory SCP exposures, 2) to measure the SCP exposure in situ and consequent responses of the benthic algal assemblage, and 3) compare exposures (in terms of initial concentration and exposure duration) and responses measured in the laboratory and in situ. Results demonstrated that laboratory exposures of H_2O_2 from SCP dissipated within 48 hours, and significant decreases in phycocyanin concentrations and densities of putative taste- and odor-producing algae were measured within 7-DAT following exposures of 453, 615, and 812 mg H_2O_2 m^{-2}. The H_2O_2 exposure measured in situ was comparable to laboratory exposures of 453, 615, and 812 mg H_2O_2 m^{-2} in terms of initial exposure (619±428 mg H_2O_2 m^{-2}) and exposure duration (dissipation within 30 hours). Comparison of measured laboratory and in situ responses provided additional evidence that comparable exposures were achieved. Significant responses in situ were measured by 7-DATin terms of phycocyanin concentrations and densities of putative taste- and odor-producing algae, and were comparable to responses obtained from effective laboratory exposures (i.e. 453-812 mg H_2O_2 m^{-2}). Decreases in measured concentrations of MIB and geosmin at the intake of the drinking water treatment facility provided additional evidence that algae were exposed to H_2O_2 from SCP. Results of this experiment illustrate the use of algaecide exposures for source water control of the production of compounds with adverse taste and odor attributes, and provide evidence for the design and use of physical laboratory models to predict organism responses in situ.

WP203 Risk posed by pesticides to native freshwater mussels in the Great Lakes Basin

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Canada supports 55 of the approximately 300 Unionidae species found in North America, with 40 species found within the Great Lakes Basin. Freshwater mussels contribute important ecological functions to aquatic systems. The water filtered by mussel assemblages can contribute to improved water quality and the filtered material deposited in sediment provides a link between pelagic and benthic food webs. In addition, mixing of sediments by burrowing mussels can improve oxygen content and release nutrients. However, nearly 70% of global freshwater mussel species are listed as either endangered, threatened, or in decline. In Ontario, 28 species are in decline or in need of protection. Even though freshwater mussels are considered extremely sensitive to contaminants, little is known about the risk pesticides pose to the most sensitive life stage: glochidia. *Villosa iris*, rainbow mussel, is currently listed as "special concern" in Ontario. A potential risk to the recovery of freshwater mussel species is the presence and persistence of pesticides and pesticide mixtures in the Great Lakes Basin. Acute (48 h) toxicity tests were performed with glochidia to determine the effect on viability following exposure to azoxystrobin, boscalid, metalaxyl, myclobutanil, carbaryl, clothianidin, imidacloprid, thiamethoxam, rotenone, malathion, prochloraz, chlorpyrifos, flupyradifurone, and cypermethrin. Glochidia were also exposed to environmentally relevant binary mixtures of pesticides to determine effects on viability. The study found that in general glochidia were relatively insensitive to the pesticides tested and LC_{50}s ranged from 594 to >17400 ug/L. All neonicotinoid LC_{50}s were greater than the highest concentration tested. The pesticides tested likely represent a *de minimis* risk to the viability of glochidia in Ontario streams where pesticide concentrations are considerably lower than those tested in this study.

WP204 Sublethal Effects of the Organophosphate Aquaculture Pesticide Azamethiphos on the Larvae of the American Lobster (Homarus americanus)

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Salmon aquaculture and the American lobster (*Homarus americanus*) industry are two economically important industries in Atlantic regions of Canada and the United States. Both industries exist in the marine ecosystem and have potential to interact. Salmosan® (active ingredient azamethiphos) is an organophosphate aquaculture pesticide used to treat Atlantic salmon for infestations of parasitic sea lice (*Lepeophtheirus salmonis*). Salmosan® is known to be lethal to adult lobster at the suggested application concentration, 100 µg/L, and a limited amount of studies have been carried out on the pesticide's effects on larval lobsters. Using 3 hour exposures to mimic pesticide plumes in seawater, this study determined that the median lethal concentration (3 h) were 5.87 ± 2.01 µg/L (stage I) and 20.45 ± 12.77 µg/L (stage IV). Surviving stage IV larvae were raised to stage V to assess sublethal effects to growth parameters. General linear model analysis ($\alpha = 0.05$) established that intermoult period, specific growth rate, and moult increment were not significantly affected by sublethal concentrations when compared to the control (< 0.05 µg/L azamethiphos). RNA sequencing was performed using Illumina Hiseq 2500 PE125. Differential expression was observed in genes transcribing proteins involved in immunity, metabolism, and stress responses such as protein degradation. Subsequent RT-qPCR was performed to confirm expression levels of genes of interest. Gene expression was used to establish effects on biological pathways of *H. americanus* in order to determine unique gene induction patterns. Established gene induction patterns may be used as a potential diagnostic tool for pesticide exposure in lobster.

WP205 Survival and biochemical health indicators of Elliptio complanata in Anacostia River tributaries for monitoring of persistent organic contaminants

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Abstract—The Anacostia River is one of three regions-of-concern in the Chesapeake Bay Watershed. Persistent organic pollutants (POPS) such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and organochlorine (OC) pesticides are known to accumulate in sediment and biota within tidal/depositional portions of the Anacostia system, but on-going contaminant sources are poorly understood. The current project investigates relative contaminant contributions to the system by deploying freshwater mussels (*Elliptio complanata*) in non-tidal reaches of six Anacostia tributaries (plus an out-of-system reference site). The large adult mussels were meant to bioaccumulate POPs during 90-day cage deployments. Filter-feeding mussels can acquire dissolved and particle-bound contaminants during feeding, making them a useful tool for monitoring total POP loads transporting through the system. Additionally, *E. complanata* are indigenous to the Anacostia River, but few individuals remain due to stream blockage in the last century restricting migration of their host fish, the American eel. A second objective of the project was to determine the suitability of various tributaries of the Anacostia River for reintroduction of *E. complanata* now that eels have few restrictions to migration. Successful reintroduction would increase benthic community diversity and potentially improve water quality in the system. Year 1 results indicate very good survival of caged mussels during 90 and 150 day deployments with only one mortality out of 336 mussels deployed. Health indices (protein and carbohydrate) of 150-d deployed mussels also suggest conditions are amendable to mussel reintroduction. Tissues from 90-d deployed mussels are currently being analyzed for POPs. Results of analyses will be presented. Preliminary results of Yr-2 deployments will also be presented.

WP206 The Effect of Different Fish Predators on Riparian Spider Contaminant Burdens

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Riparian spiders in the families Tetragnathidae (horizontal web-builders) and Araneidae (vertical web builders) feed primarily on aquatic emergent insects and have been used as bioindicators of aquatic contaminants including mercury and polychlorinated biphenyls (PCBs). However, their current use as a contaminant tracer is limited by complex aquatic interactions that can affect the %-contribution of emergent insects to their diet. These spiders have been shown to shift their diet to more terrestrial prey items when the emergence of benthic macroinvertebrates is impeded, but the effect different fish assemblages can have on the spider's dietary shift and aquatic contaminant concentration has not been explored. The objective of this study was to determine if the presence of different fish predators impact the %-contribution of emergent insects in riparian spider diets and/or their contaminant burden.To determine this, three sites in the Appalachian Mountains were selected. Two sites have a barrier separating downstream rainbow trout (*Oncorhyncus mykiss*) from upstream eastern brook trout (*Salvelinus fontinalis*) populations. The third site has sympatric populations of rainbow trout and eastern brook trout. In August of 2016, ten eastern brook trout and ten rainbow trout were collected from established stream reaches along with riparian spiders from both families. Stomachs and caudal fins were excised and remaining whole-body homogenates were analyzed for total-mercury and methyl-mercury concentrations (Upstream Eastern Brook Trout THg: 48.7 PPB ± 4.3 SE and MeHg: 45.9 PPB ± 5.4 SE; Downstream Rainbow Trout THg: 38.6 PPB ± 4.3 SE and MeHg: 37.8 PPB ± 4.3 SE). Fin clips were analyzed for stable isotopes of nitrogen and carbon and gut contents were examined. At each site, spiders were separated by family, homogenized into three samples, and then analyzed for total-mercury and stable isotopes (Upstream Tetragnathids THg: 280.7 PPB ± 63.1 SE and Araneid THg: 93.9 PPB ± 16.3 SE; Downstream Tetragnathid THg: 196.7 PPB ± 16.6 SE and Araneid THg: 110.0 PPB ± 21.7 SE). Although Tetragnathid and Araneid spiders were present at all sites, the Tetragnathid spider population was almost completely absent from the site with mixed trout populations.

WP207 Toxicity of a Metals Mixture to Early Life Stages of Crayfish Orconectes quadruncus

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Lead (Pb) and other metals resources of southeastern Missouri, USA have been exploited since the 1700s, which has left a legacy of metals-contaminated streams throughout the region. Contamination of fish and other aquatic biota, alteration of fish and invertebrate communities, and public health advisories against human consumption of Pb-contaminated fish have resulted. Crayfish are an important prey item for fish, other aquatic and terrestrial invertebrates, birds, and mammals; and they are also critical to organic matter processing in streams and the cycling of nutrients and energy through stream food webs. *Orconectes quadruncus*, listed as imperiled by Missouri and petitioned for Federal listing as an endangered species, is found in mining-impacted streams of St. Francois and Madison Counties, Missouri. Surveys conducted in the Little St. Francis River and Saline Creek found elevated concentrations of Pb, nickel (Ni), cobalt (Co), and copper (Cu) in water, sediment, and biota, and significantly lower riffle densities of *O. quadruncus* at sites directly downstream of mining areas compared to upstream sites. However, in-situ toxicity tests with caged laboratory-reared *O. quadruncus* found no significant difference in crayfish survival between sites upstream and downstream of mining-impacted sites. Laboratory toxicity tests were conducted to assess the toxicity of a site-specific metals mixture (Ni, Co, Cu, Pb, zinc, cadmium) on early (< 1-month and < 3-month) life stages of *O. quadruncus* to assess the effects of water hardness and life stage on toxicity. Chronic (28-d) toxicity tests were conducted in diluters (control plus five proportional metals mixture concentrations) in well water (290 mg/L as $CaCO_3$) and “150 hard” (150 mg/L as $CaCO_3$) water. Eight beakers containing ten crayfish were exposed in two replicates of each concentration for each water type. Mean percent survival, total length and wet weight of crayfish were determined. Provisional results indicate that site-specific metals concentrations are toxic to early life stages of *O. quadruncus* and that water hardness affects toxicity. Mean total length and wet weight data indicate similar dose-response patterns for the two water types. Results will provide resource managers with data for assessment and future restoration.

WP208 Toxicity of Water Temperature and Chlorination to Four Invasive Aquatic Plants

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The Chicago Area Waterways System (CAWS) links the Mississippi River Basin and the Great Lakes and is a potential corridor for the spread of aquatic invasive species. Because the CAWS is a potential corridor for invasive species there is interest in developing control techniques to inhibit the spread of invasive species through this corridor. A physical barrier in the CAWS would be the most effective prevention method, but the CAWS is an import shipping and boating corridor, consequently other control methods are being considered. Chemical treatment has been proposed at the O’Brien and Brandon Road Lock systems of the CAWS. A lock system may be the optimal location for chemical treatment because all boat traffic must pass through a confined area. We investigated the effectiveness of two proposed aquatic invasive species control treatments for a lock system, hot water and chlorine alone and in combination. Toxicity of hot water, five temperatures (25°C, 35°C, 40°C, 45°C, and 50°C), chlorine, four concentrations (10, 20, 50, and 100 mg Cl^-/L as sodium hypochlorite), and four exposure periods, 10, 20, 30, and 60 minutes were tested on four aquatic invasive plants species, Brazilian elodea, *Egeria densa*; parrot feather, *Myriophyllum aquaticum*; water lettuce, *Pistia stratiotes*; and European frogbit, *Hydricharis morus-ranae*. After exposure, test plants were placed in a recovery bath for 30 days to track mortality. The purpose of these tests was to determine what treatments resulted in 100% mortality for each test species. Initial results have shown that 100% mortality of Brazilian elodea was achieved at treatment temperatures of 45°C for 20, 30, and 60 minutes and all treatments at 50°C. Chlorine treatment appeared to have an impact on Brazilian elodea growth, but not on mortality. The range of temperatures and chlorine concentrations tested did not result in 100% mortality for parrot feather. Findings will be detailed for all four test species.

WP209 Toxicity Testing of Four Formulations of 2,4-Dichlorophenoxyacetic Acid (2,4-D) Herbicide at Environmentally Relevant Levels

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2,4-Dichlorophenoxyacetic acid (2,4-D) is one of the most widely used broadleaf herbicides for control of invasive aquatic plants. Although 2,4-D has been tested extensively by regulatory agencies; most of its commercial formulations have not been tested. Moreover, the concentration of 2,4-D in the sediment post-application and its effects on sediment organisms are largely unknown. This study examined two liquid amine forms of 2,4-D, Weedestroy®AM-40 (WD) and DMA®4IVM (DMA), for toxicity to the green algae Raphidocelis subcapitata, the cladoceran Ceriodaphnia dubia, and the amphipod Hyalella azteca in aqueous solutions. The midge, Chironomus dilutus, was exposed to two granular forms of 2,4-D: Navigate® (an ester version) and Sculpin G® (an amine) in a 10-day sediment test. A semipermeable membrane device called a “peeper” was used to evaluate 2,4-D concentrations in the water column and in pore water within the sediment. 2,4-D concentration in water and peeper samples was assessed by enzyme-linked immunosorbent assay (ELISA). The sediment toxicity testing done in the lab was conducted at the maximum allowed lake application rate of 4 mg/L 2,4-D acid equivalents (a.e.), while the aqueous tests had concentrations up to 32 mg/L 2,4-D a.e. Exposure to WD and DMA did not cause a significant decrease in either algal growth or survival and reproduction of C. dubia. Survival of H. azteca was also not significantly affected by either formulation. However, H. azteca growth decreased with increasing concentration of WD above 4 mg/L. C. dilutus survival and growth were not significantly affected by Sculpin® or Navigate® exposure at concentrations up to 4 mg/L. Sculpin® water column grab sample concentrations from the sediment tests were very close to 4 mg/L at test initiation but decreased over time. Navigate® grab sample concentrations were below 2 mg/L at test initiation, increased to 2.5 mg/L and did not decrease over time. Water column peeper samples were very comparable to grab samples after the first day. Sediment peeper samples ranged from no detection to 3.1 mg/L 2,4-D with an indication of some accumulation of 2,4-D in the sediment over time. The four 2,4-D formulations tested did not demonstrate significant toxicity to C. dubia, R. subcapitata, H. azteca or C. dilutus at typical surface water application rates.

WP210 Update on USEPA's Revision to the 1985 Guidelines for Deriving Aquatic Life Criteria

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USEPA's Office of Water is currently revising the 1985 *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (Stephan et al. 1985), to reflect the current state-of-the-science for aquatic effects assessments. Following a 2015 public meeting soliciting early input from the scientific community, USEPA decided to undertake two overarching parallel tracks for this revision: 1) updating and refining methods for deriving state-of-the-science criteria through comprehensive analyses, and 2) developing criteria more rapidly for the broader protection of aquatic life from the potential adverse effects of the large number of chemicals released into the aquatic environment. The first track reflects that for a smaller group of chemicals, criteria development may be scientifically complex, and deriving robust criteria for these chemicals may require detailed investigation. The second track reflects the recognition that extensive testing of all chemicals is infeasible and there is a need to efficiently derive criteria using approaches which estimate safe environmental concentrations with limited empirical data. Based on these objectives, USEPA will develop two criteria documents for this revision: 1) a Comprehensive Guidelines Document, intended to directly update and expand upon approaches presented in the 1985 Guidelines, and that will describe methods providing criteria for chemicals requiring a more detailed level of evaluation, and 2) an Expedited Guidelines Document, focusing on criteria development methods that are resource-conserving and can be used to develop scientifically-robust criteria, even when supporting data are more limited. This latter guidance will place emphasis on methods encompassing predictive tools, data extrapolation and threshold-based methods, and probability-based approaches. A Scoping Document is currently under development providing a "roadmap" to the revision process for both tracks. The Scoping Document details the objectives and approach to the revision process, and discusses the scientific and technical topic areas considered for the revision and their applicability to the Comprehensive and Expedited Guidelines documents. This poster presentation provides an update to the ongoing revision process, with emphasis on information contained within the Scoping Document, which is scheduled for release in late 2017 in support of a planned Initial Consultation with the USEPA's Science Advisory Board.

WP211 Uptake and excretion of tin in saltwort (Batis maritima) from contaminated sediments along Galveston Bay, Texas

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Tidal wetlands are among the most productive ecosystems in North America. Along the northern Gulf of Mexico, the perennial halophyte *Batis maritima* (saltwort or pickleweed) is common in salt flats associated with estuarine wetlands. As an adaptation to the high salinities characteristic of salt flat sediments, *B. maritima* shunts salts to specific leaves on the plant, which then drop off, reducing the salt concentration in the plant. In a previous study, we found that salt excretion by *B. maritima* functioned as a mechanism for reducing heavy metal burdens in *Batis* populations collected from a reference site and four sites along Galveston Bay associated with a former tin smelter and Superfund site. For the present study, the previous sites were revisited to focus on Sn uptake and excretion in *B. maritima*. At each site, a 30-m transect was established parallel to the inland edge of the marsh vegetation. Four stations were set along the transect at 10-m intervals, from which 160 green and salt leaves were collected from multiple individuals at each site. Dried leaves and sediments were acid digested and analyzed for tin concentrations by ICP. *B. maritima* absorbed and concentrated tin in both green and salt leaves. In addition, salt leaves from all plants contained significantly higher concentrations of tin than did green leaves, indicating that *saltwort* can effectively excrete tin.

Exposure and Effects of Emerging Contaminants on Aquatic Ecosystems

RP001 Aquatic sampling of PCB-11 and embryotoxicity in zebrafish (Danio rerio)

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3,3'-Dichlorobiphenol (PCB-11) is a non-legacy PCB congener primarily used in diarylide pigments for commercial printing inks, paints, sealants and plastics. Recent studies have detected PCB-11 in air, water, and sediment samples, and suggest its presence may impact water quality and health. In this study, native fish, sediment, and surface water were sampled approximately 0.25-0.5 miles downriver of a paper manufacturing facility in Western Massachusetts. Fish were obtained via electrofishing at the same time and locations as sediment and water samples. All samples were analyzed for PCB-11 at Cape Fear Analytical (Wilmington, NC) using EPA Method 1668A. Surface water PCB-11 concentrations ranged from 2.38-4.50 ng/L. Sediment PCB-11 concentrations ranged from 18-43.4 pg/g. Fish tissue concentrations ranged from 22.5-136.0 pg/g. To determine whether PCB-11 affects fish embryo development, we exposed zebrafish (*Danio rerio*) embryos at 24 hours post fertilization (hpf) to a static concentration of 0.02% DMSO or concentrations of 20 pM, 20 nM, or 20 µM PCB-11, and assessed morphology at 96 hpf. Morphometric measurements did not reveal any gross malformations associated with PCB-11 exposures. To identify interactions with either Ahr or Cytochrome P4501A (Cyp1a), we used the in vivo ethoxyresorufin-O-deethylase (EROD) assay and measured resorufin fluorescence in the gut at 96 hpf in fish exposed to either different concentrations of PCB-11 alone or in combination with a co-planar PCB, 5 nM PCB-126, a potent activator of Ahr. Our results show that the presence of PCB-11 in combination with PCB-126 significantly reduced EROD induction compared to the PCB-126 group alone in a dose-dependent manner, suggesting a potential antagonistic interaction with either Ahr or Cyp1a. Morphometric assessments of both the PCB-126 embryos and the co-exposed PCB-11+ PCB-126 embryos were all similarly disfigured with cranio-facial malformations and pericardial edema characteristic of blue sac disease. This study demonstrates that PCB-11 is present in a river in Western Massachusetts and does not appear to cause embryonic malformations in the zebrafish model at environmentally relevant concentrations. Additional studies are needed to evaluate the effects of co-exposures, outcomes at other life stages, and to identify the molecular actions of this emerging contaminant.

RP002 Assessing the lethal and sublethal toxicity of two thiocarbamates to aquatic invertebrates

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Canada's Chemicals Management Plan (CMP) was implemented by the federal government in 2006 to regulate chemicals detrimental to human health and the environment. Few toxicological data are associated with the numerous industrial chemicals listed in the CMP. Thiocarbamates are high production volume chemicals, and are imported into Canada primarily for uses related to rubber and motor vehicle parts manufacturing, although they are also used as pesticides. Despite their widespread use, there is little information on the ecotoxicity of thiocarbamates, and the research that is available focuses primarily on acute lethality. Therefore, our objective was to conduct chronic, spiked-sediment exposures to assess the toxicity of two thiocarbamate compounds, tetramethyl thiuram disulfide (TMTD or Thiram, CAS RN 137-26-8) and dipentamethylene thiuram tetrasulfide (DPTT, CAS RN 120-54-7), to three species of aquatic invertebrates: *Hyalella azteca* (amphipod), *Hexagenia* spp. (mayfly), and *Daphnia magna* (cladoceran). Three-week range-finding tests with *Hyalella* and *Hexagenia* were conducted initially, and as DPTT demonstrated little toxicity, chronic DPTT exposures were only conducted with *Daphnia*. Chronic sediment exposures were six weeks (*Hyalella* and *Hexagenia*; Thiram only) or three weeks (*Daphnia*; Thiram and DPTT), and effects on survival (all species), growth (all species), and reproduction (*Hyalella* and *Daphnia* only) were assessed. Results are currently based on nominal concentrations (mg/kg dry weight sediment), as chemical analysis of water and sediment is ongoing. Survival in Thiram exposures was significantly reduced at 100 mg/kg, with LC50s of 86, 110, and 48 mg/kg for *Hyalella*, *Hexagenia*, and *Daphnia*, respectively. Thiram also caused a significant decrease in growth of *Hexagenia* (EC50 = 69 mg/kg). Growth and reproduction of *Hyalella* and *Daphnia* were not affected by Thiram (up to 300 and 1000 mg/kg, respectively). Chronic DPTT exposures with *Daphnia* caused a significant decrease in survival (LC50 = 1000 mg/kg), but there were no significant effects on growth or reproduction (up to 1000 mg/kg). Thiram was similarly toxic to the three invertebrate species tested, with effects occurring between 48-110 mg/kg, and was 10-fold more toxic than DPTT. The results of this study will be compared to thiocarbamates measured in Canadian environmental samples, and will support environmental risk assessments to determine if thiocarbamates could impact aquatic organisms.

RP003 Assessing the toxicity of clobetasol propionate using in vitro cell assay and in vivo fish model

A.C. Mehinto, Southern California Coastal Water Research Project / Toxicology; D. Li, City of Los Angeles / Public Works; L. Mao, Southern California Coastal Water Research Project; K.A. Maruya, SCCWRP

Cell-based assays have shown promise as screening tools to assess chemical exposure and effects. Recent studies have demonstrated that cell assays can be used to assess water quality and help identify bioactive chemicals present in the water. However, the relationship between cell assay responses and adverse effects in ecologically relevant species remains poorly understood. This study investigated the toxic potential of glucocorticoids (GCs), a group of steroidal anti-inflammatory drugs widely used in human and veterinary medicine, and detected in treated wastewater effluents. Larval inland silversides (*Menidia beryllina*) were exposed to a potent GC, clobetasol propionate, at concentrations between 4 and 4,000 ng/L for 14 days. Water samples collected during the exposure were analyzed using a commercially available cell assay to determine in vitro bioactivity. The 14-day exposures did not affect survival, an endpoint frequently used in standardized toxicity testing and risk assessment. The effects on sublethal endpoints, e.g. growth (biomass and length) and molecular changes, will be presented. In addition, the linkage between in vitro bioactivity and in vivo biological changes will be characterized.

RP004 Bioaccumulation and biotransformation of sediment-bound fipronil in Lumbriculus variegatus

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Fipronil has been frequently detected in sediment. Although fipronil shows low toxicity to mammals, it is highly toxic to aquatic organisms, calling for assessing its aquatic risk. The degradation products of fipronil have the same even higher toxicity to aquatic organisms compared with the parent compound. Thus, it is better to include the toxicity of the metabolites when analyzing the toxicity of fipronil. In the current study, bioaccumulation potential of sediment-associated fipronil was evaluated using *Lumbriculus variegatus* as a model species. The biotransformation of fipronil in the blackworm was also analyzed. Three metabolites, including fipronil sulfone, fipronil sulfide and fipronil desulfinyl were detected in both sediment and organisms. Fipronil sulfone was the main metabolite in *L. variegatus*. The bioaccumulation and biotransformation processes were modeled using a first-order two-compartment toxicokinetic model.

RP006 Chlorinated paraffins in Minnesota fish: A statewide study

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Results of a previous survey of chlorinated paraffins (CPs) in Minnesota fish indicated concentrations of concern, particularly for aquatic life. These findings provided the impetus for a statewide study to determine the extent and magnitude of CP contamination in fish tissue in Minnesota. Over 350 fish representing a variety of species were collected from 44 waters in six of Minnesota's seven ecoregions. Fish from Richie Lake on Isle Royale in Lake Superior were analyzed as a point of reference. Short-, medium-, and long-chain CPs were detected in approximately 30% of the fish and approximately 40% of the water bodies tested.

RP007 Chlorinated Triclosan Derivatives and Dioxin Transformation Products: Pathways, Ecological Significance, and Risk Communication

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Consumer products contain a variety of chemicals which are discharged to domestic wastewater, and chemical transformations during treatment and after discharge can present challenges in assessing environmental impacts. Although the majority of triclosan in wastewater is removed by modern wastewater treatment, some triclosan and transformation products may be released with the effluent. Chlorinated derivatives and certain members of the dioxin family of compounds can be formed from triclosan through wastewater chlorination and ultraviolet irradiance/photolysis, and it has been speculated that these transformation products could be environmentally significant. The chlorinated derivatives of triclosan are 4-Cl-TCS, 6-Cl-TCS and 4,6-Cl-TCS. Triclosan can degrade through photolysis to form 2,8-dichlorodibenzo-p-dioxin (2,8-DCDD), a dioxin congener which does not induce enzyme activity via the aryl hydrocarbon (Ah) receptor and does not bioaccumulate in fish or other vertebrates (i.e., it is not "dioxin-like"). The chlorinated triclosan derivatives can degrade to form three additional dioxin congeners (1,2,8-TriCDD; 1,2,3,8-TCDD; 2,3,7-TriCDD), which also are not "dioxin-like." The term "dioxin" has caused confusion among researchers and the popular press, who often have failed to distinguish among dioxin congeners with vastly different toxicokinetic and toxicodynamic properties. We offer examples of such risk communication issues, and we challenge researchers and peer reviewers to examine the assumptions underlying speculative statements on the environmental and societal significance of their findings.

RP008 Comparative developmental toxicity and endocrine disruption of 6 phthalates in embryonic zebrafish assay

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Phthalates are endocrine disrupting chemicals that disturb endocrine system by similar structure with estrogen in the body. However, without careful examination, various phthalates having different molecular weights have been used in a wide range of commercial products. Zebrafish have conserved endocrine system with human, and its embryonic assay is efficient for fast screening of endocrine disruption potentials. To investigate comparative developmental toxicity and endocrine disruption effects of phthalates, zebrafish embryos were exposed to 0, 0.01, 0.1, 1.0, 10, 20 or 100 mg/L of phthalates for 168 hpf (hour post fertilization). Six phthalates, DEHP(Bis-(2-ethylhexyl) phthalate), DIDP (Di-isodecyl phthalate), DINP (Di-isononyl phthalate), DnOP (Di-n-octyl phthalate), DEP (Di-ethyl phthalate), and DMP (Di-methyl phthalate), were selected according to the prioritization considering social issues, available toxicity data and amount of usage. In embryonic developmental toxicity testing, no remarkable mortality was observed at highest concentrations of all phthalates, except DMP and DEP showing 81 and 25 % at 100 mg/L, respectively.We measured expression of genes, *Cyp19a, Cyp19b, ER-α, ER-β, lh-β, fhsr,* and *vtg1*, associated with hormone metabolism, development of yolk and corpus luteum, at 0.01, 0.1, and 1.0 mg/L. Exposure to three phthalates(i.e., DEHP, DIDP and DINP) increased the gene expression of *Cyp19b, fshr, ER-α, ER-β* in DIDP and *fshr* in DINP. DnOP exposure reduced *ER- α* at 0.01 mg/L. Our study showed that other phthalates exhibited higher potential of endocrine disruption than DEHP, known for a high endocrine disruptor among phthalates. More systematic study is required in alternatives of DEHP.

RP009 Effects of Triclosan (TCS) on fecundity, the antioxidant system, and oxidative stress-mediated gene expression in the copepod Tigriopus japonicus

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Triclosan (TCS) is an antimicrobial agent that has been widely dispersed and detected in the marine environment. However, the effects of TCS in marine invertebrates are poorly understood. In this study, the effects of TCS on life cycle history (e.g. mortality and fecundity) along with cellular reactive oxygen species (ROS) levels, GSH content, antioxidant enzymatic activities, and mRNA expression levels of oxidative stress-mediated genes were measured in the copepod *Tigriopus japonicus*. The no observed effect concentration (NOEC) and median lethal concentration (LC50) of TCS in the adult stage were determined to be 300 µg/L and 437.476 µg/L, respectively, while in the nauplius stages the corresponding values were 20 µg/L, and 51.76 µg/L, respectively. Fecundity was significantly reduced ($P < 0.05$) in response to TCS at 100 µg/L. Concentration and time-dependent analysis of ROS, GSH content (%), and antioxidant enzymatic activities (e.g. GST, GPx, and SOD) were significantly increased ($P < 0.05$) in response to TCS exposure. Additionally, mRNA expression of detoxification (e.g., CYPs) and antioxidant (e.g., glutathione S-transferase-sigma isoforms, Cu/Zn superoxide dismutase, catalase) genes was modulated in response to TCS exposure at different concentrations over a 24 h period. Our results revealed that TCS can induce reduced fecundity and oxidative stress with transcriptional regulation of oxidative stress-mediated genes with activation of the antioxidant system in the copepod *T. japonicus*. Overall, TCS affects survival through oxidative stress with antioxidant and detoxification defense system in *T. japonicus*. In addition, two *CYP* genes (*CYP3026A3* and *CYP3037A1*) are likely to have a potential as biomarkers in response to TCS in *T. japonicus*. This study will be helpful for a better understanding of how TCS affects on antioxidant defense and detoxification mechanisms in copepod.

RP010 Exposure to the gestagens progesterone and levonorgestrel alter the growth and mobility of juvenile apple snails (Pomacea canaliculata)

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Recent studies examining the impacts of gestagen exposure on aquatic wildlife have been shown to cause decreases in the reproductive fitness of multiple aquatic species. Gestagens, which include the mammalian steroid hormone progesterone (P4) and the synthetic progestin levonorgestrel (LNG), have been detected in runoff from agricultural fields and as a component of wastewater treatment plant effluent in low ng/L concentrations. While both of these endocrine disrupting chemicals (EDCs) have been shown to alter fecundity, morphology, and reproductive behavior in fish, their effects on invertebrates are poorly understood. The invasive freshwater snail *Pomacea canaliculata* (Ampullariidae) is sexually dimorphic and often inhabits environments that are prone to contamination, making it a suitable model for studies examining the impacts of EDC exposure. This species has previously been used to examine the impacts of tributyltin and testosterone on reproduction. In this study, juvenile *P. canaliculata* were exposed to environmentally relevant concentrations of P4 and LNG, and differences in growth and mobility assessed. Snails (n=15) were exposed for 25 days to either an ethanol control, 30 ng/L P4, 300 ng/L P4, 10 ng/L LNG, or 100 ng/L LNG using static replacement (full change every 24hrs). Snails were fed *ad libitum* and maintained at standard photothermal conditions. To assess growth, shell height was measured on days 14 and 25, and locomotion for each population was assessed using behavioral analysis software on day 14. Snails exposed to both P4 and LNG differed significantly from the control group ($p < 0.05$) in both growth rates and activity levels, suggesting that P4, LNG, and other related EDCs may be negatively impacting the viability of wild populations exposed to gestagen-polluted environments.

RP011 Healthy fish, healthy people: Ecological and human health impacts of early life exposures to endocrine disruptors on metabolic and bone development

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Fish inhabiting the New Bedford Harbor (NBH), Massachusetts, marine Superfund site can serve both as biological models for contaminant effects and indicators of human dietary exposure, contributing importantly to the assessment of ecological and human health risks of contaminant exposure. Polychlorinated biphenyls (PCB) and tributyltin (TBT) are bioaccumulative contaminants associated with NBH industries that belong to a growing class of metabolism-disrupting compounds believed to contribute to obesity, liver steatosis, and Type 2 diabetes in humans and other species. Here we show that embryonic exposure to PCBs and TBT produced phenotypic abnormalities and altered the expression of genes related to metabolic homeostasis in laboratory-reared killifish (*Fundulus heteroclitus*), an ecologically-important NBH fish. These biological effects suggest perturbations to metabolic and bone homeostasis in fish, consistent with effects seen in mammalian species; future transcriptomic analyses will provide insight into the underlying molecular mechanisms of toxicity for these compounds in fish. In a complementary investigation, we also used information from NBH seafood as a proxy for human dietary exposure to harbor-based Superfund chemicals, and show here that PCBs in human-consumed species from NBH have generally declined since 2003. This information may be useful in understanding the contribution of chemical metabolic disruptors in human obesity and metabolic disease. The combination of mechanistic studies using fish and the assessment of potential human exposure through consumption of contaminated seafood provides an effective and holistic approach to characterize both ecological and human health risks of exposure to environmental chemicals, including those frequently found at sites highly contaminated with multiple Superfund chemicals.

RP012 Impact of carbon nanomaterial physical properties on co-exposure with benzo(a)pyrene in fish liver cells

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Engineered carbon nanomaterials of various shapes, sizes, and surface chemistries are under development for a wide range of commercial applications that could result in release to aquatic environments. Many waterways in the United States are already contaminated with pollutants like aromatic hydrocarbons that may interact with released carbon nanomaterials in ways that alter their bioavailability and toxicity. Using the fish liver cell line, PLHC-1, we are studying the environmental impacts of co-exposure to polycyclic aromatic hydrocarbons and carbon nanomaterials. Comparison of two-dimensional (2D) few-layer graphene and spherical carbon black will allow us to contrast the role of shape and surface characteristics on carbon nanomaterial interactions with the model aromatic hydrocarbon benzo(a)pyrene. Preliminary results suggest that both shape and surface area play critical roles in this interaction, and we are now focused on delineating how the different particle properties effect the overall toxicity of this mixture both in acute and subchronic exposures in vitro. By integrating morphological and molecular markers of benzo(a)pyrene toxicity in fish liver cells, we will be able to describe how nanomaterial characteristics effect the bioavailability and toxicity of adsorbed benzo(a)pyrene. Together, this study will allow us to determine the impact of carbon nanomaterial properties on their interactions with aromatic hydrocarbons and the potential implications of this interaction on cellular adaptive and toxic responses. This research is supported by NIEHS Superfund Research Program P42 ES013660, the Institute at Brown for Environment & Society, and the generous support of Donna McGraw Weiss ’89 and Jason Weiss.

RP013 Male Fathead Minnows Exhibit Decreased Sperm Motion Characteristics and Increased Nest Acquisition Success Due to Levonorgestrel Exposure

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Synthetic progestins are utilized as a component of human contraceptives, and enter the environment as a component of wastewater treatment plant effluent. Certain progestins have been shown to activate fish androgen receptors, resulting in the morphological masculinization of females, changes in reproductive behaviors, and decreased egg deposition. In this study, a nest acquisition assay and computer assisted sperm analysis (CASA) was used to examine the effects of levonorgestrel (LNG) on male fathead minnow (*Pimephales promelas*) reproductive fitness. Males were exposed to 0, 10, or 100ng/L LNG for 14d. Combinations of a control male and a male from one of the treatments were placed into a competitive nesting assay, and time each male spent holding the nest and time spent exhibiting aggressive behaviors were analyzed at 8, 24, and 48hrs post-exposure. Semen samples were analyzed for total motility, straight-line velocity, curvilinear velocity, average path velocity, linearity, beat cross frequency, and wobble at 0, 30, 60, 90, and 120 sec post-activation. Males exposed to either concentration of LNG exhibited increased nest acquisition success and lower levels of aggression compared to control-control pairings, as well as decreases in multiple motion characteristics. Results suggest a synergistic effect between these two endpoints that could have profound effects on wild populations of fish exposed to LNG and other similar progestins.

RP014 SSRIs in WWTP effluents and their disposition and effects in salmonids and marine flatfish

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In the Puget Sound region, more than 90% of municipal wastewater treatment plant (WWTP) effluents are directly released into the marine environment. We surveyed eight WWTP effluents for six different SSRIs and found total levels varied between 77 and 1,673 ng/L. The most abundant SSRIs (>90% of ∑SSRI) detected were citalopram >>> fluoxetine > sertraline. We subsequently performed a series of in vivo and in vitro exposures to assess the uptake, metabolism and effects of these SSRIs both as a mixture (at ratios observed in effluents) and as individual chemicals in salmonids and English sole. Static water exposures to an SSRI mixture in rainbow trout revealed sertraline was the most rapidly absorbed SSRI with an uptake clearance of approximately 35 ml/hr/g, nearly 10x more rapid than fluoxetine. Citalopram was the least absorbed SSRI. Subsequent continuous exposures of trout and English sole to a similar SSRI mixture indicated the kidney>> liver > brain were the tissues that accumulated the highest concentrations of SSRIs. Substantial formation of the sertraline metabolite norsertraline was observed in both trout and sole. Concentrations of norsertraline were similar or higher than sertraline in some tissues such as the liver and brain. High levels of sertraline and norsertraline were found in bile, which increased three fold after enzymatic deconjugation. In vitro studies using hepatocytes or liver homogenates confirmed sertraline is the most rapidly metabolized SSRI. Additional in vitro studies using primary pituitary cells and isolated ovarian follicles found the most sensitive effect of SSRI exposure was antagonism of the estrogen induced expression of the beta subunit for luteinizing hormone. These results suggest sertraline is the SSRI most likely to bioaccumulate and become biotransformed by fishes and achieve tissue levels near the threshold for biological effect. Supported by USEPA-STAR grant R835167, WA Dept. of Ecology G1400206.

RP015 Synthetic fragrances and organic flame retardants in fish and water from the Passaic River, New Jersey

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The lower Passaic River is a highly industrialized tributary of the Hudson River system that has been subjected to heavy industrialization and urbanization, resulting in severe degradation of the local aquatic environment. While several studies have contributed valuable information on legacy pollutants in this region, there is little information about currently and recently used emerging contaminants that may act as additional stressors on aquatic life in the lower Passaic. In this study, polycyclic musks (PCMs) and several novel halogenated flame retardants (NHFRs) were measured in polyethylene passive samplers (PEs) deployed in river water, and in biota collected from the lower Passaic from 2011 to 2012. 350 specimens of 10 different species were collected from three sites along the Passaic. The objectives of the study were to provide baseline concentrations of these contaminants in regional food webs and calculate lipid-normalized bioaccumulation factors (BAFs) using PE-derived truly dissolved water concentrations. Dissolved total PCMs (Σ_5PCM) in water ranged from 10-26 ng/L, with >98% contributed by the two most widely used PCMs, HHCB and AHTN. Total concentrations in biota ranged from 146 ng/g lipid in Pumpkinseed fish (*lepomis gibbosus*) collected above Dundee Dam to 146 ng/g lipid in silvery minnow (*hybognathus regius*) from a site downstream of the dam. BAFs were generally greater than previous estimates available for similar species, possibly due to lower water concentrations resulting from the use of PEs, which select for the truly dissolved phase. Several bromobenzenes, including pentabromobenzene (PBBz), hexabromobenzene (HBBz), and pentabromotoluene (PBT), as well as other organic flame retardants, including anti- and syn-Dechlorane Plus, were detected in the majority of biota. Concentrations of Σ_8NHFR in aquatic biota ranged from 296 ng/g lipid in striped bass (*morone saxatilis*) to 1950 ng/g lipid in Gizzard shad (*dorosoma cepedianum*). Results for these currently used contaminants will be compared to distributions of legacy contaminants in the same samples, and the importance of local population centers, combined sewer overflows, and wastewater treatment facilities in influencing spatial distributions of PCMs and NHFRs in the waterway and biota will be discussed.

RP016 Thyroid Disrupting Effects of Halogenated and Next Generation Chemicals on Developing Fish

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Numerous halogens act as endocrine disrupting chemicals (EDCs) that can alter thyroid function. Members of this group include perfluorinated chemicals, commonly used in Teflon and food packaging, and flame retardants, used in a wide array of products such as clothing and electronics. Exposure of organisms to these EDCs can negatively affect growth and development. Therefore, there is a need to develop next generation, less toxic chemicals. The objective of this study is to further test these potential "safer" alternatives for EDC activity. We used zebrafish embryos as our model organism due to its high structural and functional homology to vertebrates and its suitability for early developmental studies. Three well known hazardous EDCs, perfluorooctanoic acid (PFOA), tris (1,3-dichloro-2-propyl) phosphate (TDCPP) and tetrabromobisphenal A (TBBPA), and two next generation chemicals, 9,10-Dihydro-9-oxa-10-phosphaphenanthrene 10-oxide (DOPO) and perfluorobutyric acid (PFBA), were tested for the potential thyroid disruptive potential. We measured effects on swim bladder development as a sensitive endpoint of thyroid toxicity. Specifically, we quantified changes in the surface area of the swim bladder as well as changes in expression levels of genes involved in thyroid dysregulation. Our findings suggest developmental delay in fish exposed to sublethal concentrations, 1% of the lethal concentration required to kill 50% of the population (LC_{50}) of all chemicals tested; however, toxic mechanisms are unclear. Both acute, 6 days post fertilization (dpf), and chronic, 28dpf, studies were conducted. We further tested this finding by using Japanese medaka and reported SB over inflation, but only in females, strongly suggesting gender differences in SB dysfunction.

RP017 Integrated molecular approach in C. fluminea exposed to nCeO2: A mesocosm study

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Nanotechnology interest has considerably grown during last decades and nanoproducts are now part of our everyday life. Nanoparticle (NP) concentrations in environment are not yet known but releases during their life cycle are an evidence. NP harmful effects have already been demonstrated on aquatic species but their potential toxicity at more realistic low exposure concentration is still not well studied. Cerium dioxide nanoparticles ($nCeO_2$) are listed in OECD priority list of manufactured nanomaterials for their assessment because of their large production making them available for widespread uses in daily products. One important utilization occurs via their incorporation in diesel fuel additive due to their oxidative capacities making them useful to reduce fuel consumption, improving its combustion and reducing soot in exhaust gas. Several studies highlighted that $nCeO_2$ impact organism's physiology by affecting oxidative defenses and cellular membrane stability while gene expression studies remain scarce especially among non-sequenced organisms. The aim of this work was to follow gene expression in a widespread freshwater bivalve, *Corbicula fluminea*, exposed in indoor mesocosm to multistress conditions. Two salinities (1.5 and 15 PSU) were applied to cover a range of salinity from the continent to the estuaries and three NPs were selected in order to have both standardized and commercial NPs but also to mimic changes occurring after NP uses. Two studies were done on variation of 12 gene expression by RT-qPCR in gills and digestive gland according to the exposure conditions and time. First, a comparison of these 12 genes basal expression level in control gills and digestive gland was done and revealed strong differences of expression level (>2 fold) for some genes. The second study was about global profile of gene expression responses according to salinity and contamination. They are clearly different according to the salinity and both organs selected were responding to NP contamination. Results obtained for NPs extracted from commercial product have highlighted the need of further studies using consumer available NPs.

More Data is Not Always Better – Using Weight-of-Evidence Approaches in Environmental Risk Characterization

RP018 The effects of ionizing radiation on mutation rates: A meta-analysis of variation across studies

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The analysis on the effects of ionizing radiation plays a large role on the mitigation of effects after contamination and exposure. Studies on the effects of ionizing radiation on mutation rates have greatly differed in their estimates likely due to differences in methodologies and underlying biological variation. A meta-analysis was applied to 40 of these studies in efforts to understand the variation in the effects of ionizing radiation on mutation rates. On one side, the study focused on determining the efficiency of various molecular techniques and study designs for detecting changes in mutation rate. On the other, it analyzed biological variation in mutation rates as a function of the cell line and species used. The effects of ionizing radiation on mutation rates were determined for each study by extracting statistics and converting them to the same effect size, which provided a direct and comparable measure of the magnitude of these effects. In this meta-analysis, thirteen of the studies tested for effects on mutation rate through minisatellite markers, ten via Expanded Single Tandem Repeat analyses, eight via specific locus sequencing, three via microsatellite markers, and one via RAD sequencing. Five additional studies inferred mutation rates from phenotypic observations. Studies ranged from laboratory studies (32) to field studies (8) conducted after nuclear disasters such as the Chernobyl nuclear accident and the Fukushima Daiichi nuclear accident. These studies also varied in radiation dose and dose rate, as well as the type of radiation. Species ranged from lab mice to humans, spanning one to three generations. Our findings point to the optimal detection method for changes in mutation rate induced by ionizing radiation. They will also be useful to understand variation in susceptibility to this mutagen as well as for choosing sensitive indicator species in environmental assessment.

RP019 The Importance of Weight-of-Evidence Ecological Risk Assessment in Brownfield Redevelopment: New Jersey Case Study

B.S. Yates, Amy S. Greene Environmental Consultants, Inc. / Environmental Science; R. Britton, Whitman

The purpose of this presentation is to outline the lines of evidence used to identify ecological risks associated with the conversion of a 65-acre manufacturing facility (brownfield) to a commercial/retail development. In 2010, Whitman was contracted by a well-known manufacturing company to conduct an environmental investigation of their property located in central New Jersey. Whitman identified over 100 potential areas of concern (AOCs) including a stormwater drainage system, fire pond, and former debris disposal area with the potential to impact three on-site waterways and associated terrestrial wetland soils. Soil, sediment and surface water sampling conducted by Amy S. Greene Environmental Consultants, Inc. (ASGECI) confirmed exceedances of the NJDEP Ecological Screening Criteria for bis(2-ethylhexyl)phthalate, heavy metals and polycyclic aromatic hydrocarbons. The cost of remediating waterway sediments and terrestrial wetland soils to the NJDEP screening criteria was estimated to be 4 to 6.5 million dollars, and would require extensive disturbance of floodplain forest. Therefore, an ecological risk assessment was initiated. Working with the New Jersey Department of Environmental Protection (NJDEP), a sampling plan was developed which involved collection of pore water, fish and benthic invertebrate samples. Based on NJDEP recommendations, impacts to the floodplain were also delineated and evaluated by conducting earthworm toxicity tests, an invertebrate community survey and sampling of vegetation, berries, foliage, and gastropods. The results of these studies indicated limited bioavailability and/or bioaccumulation across most of the waterways and floodplain. While earthworm toxicity test results were highly variable, community indices indicated that even highly contaminated floodplain soils were supporting large numbers of earthworms, gastropods, springtails, beetles and other arthropods. Contaminated "hot spots" within the floodplain were identified as locations with elevated bioavailability, as measured through earthworm bioassays and/or the absence of soil invertebrates with increasing heavy metal concentrations. In conclusion, our case study demonstrates how interpreting ecological risks through disparate lines of evidence (e.g. toxicity tests and community surveys) can streamline the remedial process and facilitate the revitalization of a highly desirable property.

RP020 The potential for 6:2 FTOH to modulate the endocrine system in wildlife: A hypothesis driven weight of evidence analysis across endocrine pathways

D. Huggett, EAG Laboratories; S. Harvey, ToxStrategies, Inc.; S. Korzeniowski, BeachEdge Consulting; S. Borghoff, ToxStrategies, Inc.

6:2 Fluorotelomer alcohol (6:2 FTOH) is primarily manufactured for use as a raw material in fluorotelomer surfactants and side-chain fluorotelomer monomers (i.e. acrylates/methacrylate) production. To evaluate if 6:2 FTOH has any potential endocrine activity, a hypothesis driven weight of evidence (WoE) analysis was conducted to evaluate the potential activity of 6:2 FTOH with the estrogen (E), androgen (A), thyroid (T) and or steroidogenesis (S) pathways. This WoE assessment involved consideration of weighting endpoints by 3 rank ordered categories (Rank 1, 2 or 3) (Borgert et al., Birth Defects Res 2014. 101:90) per pathway as measured in Level 1 to Level 4 studies within the OECD Conceptual Framework for assessing endocrine disruptors (ED). Following a comprehensive literature search using Pubmed and Embase followed by hand-searching and evaluation of secondary sources, a total of 18 in vitro and in vivo studies across species were identified for review. Data available in the High Throughput Screening (HTS) ToxCast/Tox21 database were also included in this assessment. The studies identified were reviewed for reliability (ToxRTool) and relevance with endpoints extracted, ranked, and evaluated across lines of evidence per pathway. Overall, the 6:2 FTOH data suggest no interaction with the A, T or S pathways. Regarding the E pathway, there was no evidence of activity in mammalian in vivo studies. There is conflicting information with respect to E receptor activation results in the peer-reviewed literature versus the HTS assay data where the peer-reviewed data suggest the possibility of weak E activity. There was limited evidence for E activity in 3 non-guideline wildlife studies, where the only validated E endpoint measured was vitellogenin level. Based on the available data, 6:2 FTOH does not appear to modulate the A, T or S pathways; however, this limited published data suggests that the E pathway warrants additional research.

RP021 The potential for PFHxA to modulate the endocrine system in wildlife: A hypothesis driven weight of evidence analysis across endocrine pathways

S. Borghoff, S. Harvey, ToxStrategies, Inc.; S. Korzeniowski, BeachEdge Consulting; D. Huggett, EAG Laboratories

Perfluorohexanoic acid (PFHxA) is an environmental degradation product of C6-based fluorotelomer intermediates, used to produce various kinds of polymers. Considering the potential endocrine activity of perfluoroakyl acids, such as PFOA, a hypothesis driven weight of evidence (WoE) analysis was conducted to evaluate the potential endocrine activity of PFHxA, as defined by WHO, across the estrogen (E), androgen (A), thyroid (T) and steroidogenesis (S) pathways. This WoE assessment involved consideration of weighting endpoints by 3 rank ordered categories (Rank 1, 2 or 3) (Borgert et al., Birth Defects Res 2014. 101:90) per pathway as measured in Level 1 to Level 4 studies within the OECD Conceptual Framework for assessing endocrine disruptors (ED). Following a comprehensive literature search using Pubmed and Embase followed by hand-searching and evaluation of secondary sources, a total of 21 in vitro and in vivo studies across species were identified for review. Data available in the High Throughput Screening ToxCast/Tox21 database were also included in this assessment. The studies identified were reviewed for reliability (ToxRTool) and relevance with endpoints extracted, ranked, and evaluated across lines of evidence per pathway. Overall, PFHxA showed no endocrine effects in juvenile rainbow trout, and no effects across thyroid axis endpoints in chickens or reproductive parameters in Northern bobwhite quail (OECD Guideline 206). In rat studies, there was no significant activity associated with PFHxA exposure in endocrine endpoints in repeat-dose toxicity, a lifetime cancer, or guideline (OECD 422) and non-guideline reproductive and developmental studies. *In vitro* assays indicated weak to negative activity for T hormone transport protein or T hormone activation, with relatively no binding or activation of the E or A receptors. Along with negative endpoint activity evaluated in rat studies, PFHxA was negative for steroidogenesis in vitro. Based on this hypothesis-driven WoE assessment across E, A, T, and S pathways, the evidence base demonstrated that exposure to PFHxA does not induce adverse effects in various tissues of the endocrine system, has only a mild effect on various hormone levels, either shows no binding or binds weakly to hormone receptors in vitro, and does not cause any adverse effect associated with ED. As such, exposure to PFHxA would not be expected to produce an effect on endocrine systems based on the WHO definition of an ED.

Environmental Data Mining – Doing Research With No Money

RP022 Solving the data puzzle: Using cross-media data to perform cumulative environmental risk assessments

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In the United States many state agencies are required or directed to collect environmental data by the federal government; or are directed to collected environmental data by their state legislature or local stakeholders. Environmental data collected by government agencies. These data sets include measured concentrations, modeled emissions for non-point and point sources at frequencies that are continuous, integrated or one-time grab samples. Government data include long-term monitoring and collection, or shorter term monitoring from special projects or grant funded studies. Furthermore, these data span the range of environmental media (e.g. sediment, air, surface water, soil, biota) and are stored in separate databases, making it difficult for agency analysts to know what data exist outside of the environmental media of their expertise. In all of these circumstances, generally more fiscal and personnel resources are directed to the collection of these data than for their statistical analysis and summarization. This provides both scientists and decision makers with intriguing opportunities, but also challenges to pull all of this information together in order to properly describe an environmental system. Furthermore, pulling together diverse data sets is critical when addressing broad system-wide questions such as impacts within an entire airshed or watershed. One of the broad issues environmental and public health agencies face is the fact that many regulatory analyses, specifically human health risk assessments, do not include many (and never all) of the possible components of a cumulative impacts analysis. These components include pollutants from multiple sources, pathways, and from multiple environmental media. Analyses that are cumulative in nature assist agencies in making science-based decisions with important contextual information. This poster covers a case study project in which we set out to determine if our agency had measurements for the same pollutants during similar timeframes for multiple environmental media. The chemical (PFOS, Perfluorooctane sulfonate) was chosen to develop a system to pull together multiple disparate data sources for use in an aggregate risk assessment and mass balance-based modeling efforts.

RP023 The decadal variation of PAH concentrations in sediments at Osaka Bay, Japan: Usage of ChemTHEATER as database for environmental contaminants data

S. Uno, Kagoshima University / Faculty of Fisheries; E. Kokushi, Kagoshima University / Education and Research Center for Marine Resources and Environment

Until now, there have been countlessly the monitoring data for chemical contaminants. However, most of them perhaps ineffectively utilized, because their data were sometimes reported only a part without supplemented data and difficult to find the required data effectively. ChemTHEATRE (http://chem-theatre.com/) is the platform to deposit and visualize monitoring data of environmental contaminants. ChemTHEATRE describes that this database will ensure traceability of chemicals and help to simulate the environmental behavior and fate, or assess the risk. We investigated PAH concentrations and distributions in sediments at 26 sites in Osaka Bay, Japan from 2014 to 2016. In this investigation, we could find specific distribution patterns for 18 individual PAHs and also heavy polluted sites. However, it's difficult to determine whether these were temporary or persistent, and PAH pollutions improved or became quite significant. Therefore, we try to estimate the decadal variations of PAH concentrations and distributions in sediments at Osaka Bay. In this estimation, we referred the PAH data at Osaka Bay around 2000-2006, which are recorded in ChemTHEATER. We will visually show the variations with distribution map.

RP024 Statistical Evaluations of PCB Stormwater Background for a Large Federal Facility

R.T. Ryti, W.L. Swanson, Neptune and Company, Inc.; T.L. Kostrubala, A.B. White, A.R. Groffman, S.J. Veenis, Los Alamos National Laboratory / Environmental Programs

Los Alamos National Laboratory and the New Mexico Environment Department have collected storm water for Clean Water Act compliance since 2000. Some of the parameters being measured (e.g., aluminum) have no apparent site source and are likely related to background. Other parameters have both background and site sources (e.g., PCBs). This paper presents statistical comparisons of the site data population to both natural and developed background populations. The locations where natural background is a significant contributor were identified in 2012. It is likely that other natural background locations exist, and improved assessments of background contributions to storm water would be possible with a larger set of such locations. Development is assessed using information on land cover types, the presence/absence of roads, and the presence/absence of source areas of constituents such as copper sourced from developed landscapes. We also evaluate the homolog and congener data from EPA Method 1668 using a variety of multivariate data mining techniques to help determine the source of the PCBs. These statistical analyses, taken in concert with the conceptual model for PCB distribution in the environment, identify sample locations that are associated with background. This is report LA-UR-17-24796 of the Los Alamos National Laboratory.

Ecosystem Services, Stakeholder Values and Sustainability

RP025 Using an ecosystem services approach to manage sedimentation issues in an urbanized watershed and sensitive coastal lagoon

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The Los Peñasquitos Lagoon, is one of the few remaining salt marsh lagoons in southern California providing habitat for a variety of plants and animals, some of which are endangered, as well as being an important stopover for migratory birds. The Lagoon receives freshwater inputs from approximately a 60,000-acre watershed that has become highly developed. Sedimentation in the lagoon has been identified as a significant factor impacting beneficial uses in the lagoon and runoff from urban development has been identified as a leading source of sedimentation. Increasing urban development has also resulted in alterations in hydrology and modified geomorphic conditions within the three main tributaries of the Lagoon's watershed, resulting in excess sedimentation. To meet the current TMDL, we calculated that a 67% reduction in sediment loading would be required to meet historical (natural) sediment load estimates. To address this problem, an ecosystem goods and services (EGS) approach is being used to engage stakeholders and identify cost-effective and holistic solutions that will enhance or restore historic uses of the watershed and the lagoon while providing many co-benefits. Using both InVest and modifications of EPA's EnviroAtlas, we identified critical areas of the watershed in which sediment load reductions would result in the greatest EGS in the watershed overall and in the lagoon. This analysis indicated ways to increase flood control and reduce soil erosion and thereby decrease sediment loading while providing other habitats in the watershed. These results are being informed by stakeholder goals and several low-cost management options are being evaluated based on the EGS results.

RP026 Long Term Records of Lake Clarity as an Indicator for Final Ecosystem Goods and Services of Lakes

T. Hollenhorst, USEPA

We reviewed available long term records of lake clarity (via secchi disc readings) as an indicator of final ecosystem goods and services of lakes.

RP027 Using population models to gain insights into direct and indirect effects of pesticides on listed fish populations

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The U.S. Endangered Species Act has the goal of protecting the continued existence and diversity of species as part of the natural heritage of the nation. The law recognizes this ecosystem service provided by endangered species that may be valued for cultural, aesthetic, recreational or other reasons. The protection goal for listed species is generally the long-term survival and recovery of species populations. Ecological models provide a tool to evaluate this protection goal as part of the total services provided by an ecosystem. We present a population model for the threatened Slackwater darter (*Etheostoma boschungi*) to identify stressors and assess levels of stress that may affect population decline. The model describes Slackwater darter population trends by considering indirect effects of stressors on the food web and food availability. Using readily available information in the published scientific literature, we incorporated relationships between reduced food availability and body size, survival, and fecundity in fish into the Slackwater darter model. We analyzed exposure-effects relationships of a pesticide with the model to estimate exposure levels that could cause long-term effects on population growth and abundance. Further, we assessed the applicability of the modeling approach to a second listed fish species to explore the application of a species-specific model to related species with similar life histories. By combining information on life history and direct and indirect effects, population models can provide a valuable tool to assess potential risks of pesticides to populations of listed and other non-target species over ecologically relevant time periods.

RP028 Effects of metal mixtures goes beyond toxicity to soil microbes and invertebrates

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Structural equation modeling (SEM) is a powerful statistical tool that takes a confirmatory approach in analyzing structural data based on a phenomenon. In this study SEM's are being employed to help us understand how theoretical causal relationships between physico-chemical properties of soils and the benefits we derive from soils change when a site is impacted. Due to the complex nature of these relationships and networks in soil ecosystems, these alterations are usually difficult to capture in complex data. To achieve this, 47 Canadian soils covering a wide range of soil properties were collected from Alberta, Manitoba, Saskatchewan and Ontario. Each of these soils was spiked with a single dose of a metal mixture containing cobalt, copper, lead, nickel and zinc. Endpoints that represented climate regulation, nutrient cycling, xenobiotic degradation, organic matter decomposition, and forage quality were measured in all 47 soils. For climate regulation, CO_2, CH_4, N_2O gas production and consumption was measured. For nutrient cycling, the activities of the soil enzymes, ammonia monooxygenases, glucosidases and acid phosphatases were measured. For xenobiotic degradation, the soils were dosed with ^{13}Clabelled glyphosate, and the degradation rate was determined by a measure of mineralized $^{13}CO_2$. For organic matter decomposition, survival and reproduction of *Oppia nitens* and *Enchytraeus crypticus* exposed to the soils were recorded. For forage quality, crude protein content of *Elymus lanceolatus* was measured after 35 days of seeding. Results showed that, apart from the inhibitory effects that sub lethal metal mixture concentrations have on soil life(services), the ability of soils to support ecosystem services are reduced especially for organic matter decomposition and climate regulation.

Aquatic and Terrestrial Plants in Ecotoxicology and Risk Assessment

RP029 A proposed ring-test protocol for the emergent sediment-rooted macrophyte, Glyceria maxima in a water-sediment system

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Under EU pesticide regulation, regulatory tests are required for the aquatic macrophyte, *Lemna*, and two algal species for herbicides and plant growth regulators. Data requirements introduced under EU Directive 1107/2009 stipulate that further tests may be required for compounds which show selectively higher toxicity to either dicotyledonous or monocotyledonous plant species in terrestrial plant tests. In these cases, the recommended dicot and monocot species are *Myriophyllum spicatum* and *Glyceria maxima*, respectively. OECD Test Guideline 239 for testing *Myriophyllum spicatum* in a water-sediment system was adopted in September 2014. The general principles of this test system are applicable to many aquatic plant species and, in 2014, a workgroup was formed to facilitate adaptation of this protocol for testing the emergent, reed grass, *Glyceria maxima*. Since this time, 15 laboratories have expressed their interest in participating in a ring-test of this protocol and completed a survey of intended plant propagation methods. Results of this survey confirmed difficulties with the use of seedlings as test material, such that the protocol now recommends use of rhizome-propagated plants. The workgroup has identified two test substances (herbicides) and laboratories have started tests with the first herbicide in Autumn 2016. The first series of tests was designed to evaluate whether a test duration of 14 or 21 days is required and to gain information regarding control variability across several assessment parameters, i.e. shoot height, leaf length, fresh weight and dry weight. Preliminary data show that shoot height is an endpoint that is accompanied by the highest variability (highest Coefficients of Variation (CVs)). Preliminary data also show that there is a need to restrict the variability and size of the plants at test initiation. After evaluation of all ring-test results, the duration of the experiment will be set based on the doubling time and the impact of test duration on the CVs. More results will be explained on the poster.

RP030 Plant Uptake of Selected Contaminants of Emerging Concerns (CECs) Under Current Atmospheric and Elevated CO2 Conditions

H. Zhi, C. Muerdter, G.H. LeFevre, University of Iowa / Civil and Environmental Engineering

Improving the sustainable use of water in planted systems, such as bioretention or agricultural irrigation with recycled water, requires better understanding of the behavior of contaminants of emerging concern (CECs) in plants. Many CECs that interact with plants in the environment, such as biocides and fungicides, are complex organic nitrogen-containing species. The current study evaluated the plant uptake rates and transformation products of selected nitrogen-containing CECs: 5-methyl-1H-benzotriazole, benzimidazole, and isothiazolinones. Because nitrogen-containing CECs have structural similarities to amino acids, elevated CO_2 conditions are likely to increase plant assimilation of these compounds, thus increasing human exposure potential. Therefore, this study also evaluated the assimilation of 5-methyl-1H-benzotriazole and benzimidazole by plants under elevated CO_2 conditions. Although plants under elevated CO_2 conditions exhibited greater biomass, there was no difference in plant uptake of selected compounds between elevated CO_2 conditions and atmospheric conditions ($p > 0.05$). One possible reason was that the selected CECs concentration (30 µg/L) was below the uptake capacity of *Arabidopsis* plants under both ambient and elevated CO_2 conditions. For benzotriazole and benzimidazole, both glycosylation and tryptophan synthesis occurred during rapid phytotransformation. This study helps further understanding of the biotransformation of CECs in the environment, understand the potential for consumer CECs exposure from plants, and improve the efficacy of engineered natural treatment systems and water reuse.

RP031 Effect of copper oxide nanoparticles and arsenic on seed germination and seedling growth of rice (Oryza sativa japonica)

J. Liu, Baylor University; B. Dhungana, Baylor University / Chemistry and Biochemistry; G.P. Cobb, Baylor University / Environmental Science

Elevated Arsenic (As) concentration in soil and water bodies has not only decreased rice production, but has also become a serious human health concern due to the consumption of As-contaminated rice. Increased production of copper oxide nanoparticles (nCuO) and its application in agriculture may pose an additional risk to rice. The influence of nCuO on As uptake in and effects on rice are also considerations. In this study, rice (*Oryza Sativa japonica*) seed germination and seedling growth were investigated in sand media containing nCuO and As, separately or in combination. Rice was grown in an incubator for 18 days. Twelve treatments were used: control, As in sand (10 mg/kg sand), nutrient solutions containing one of five nCuO concentrations (0.1, 1.0, 10, 50, and 100 mg/L) and five As + nCuO treatments. Both treatment and time of exposure significantly decreased germination (p-value < 0.01). Rice seedling growth (i.e., length of shoot and root) decreased in all treatments compared with the control. The As treatment caused the most severe inhibition of seed germination and seedling growth, especially root growth. The addition of nCuO mitigated the adverse effects of As on seed germination and seedling growth. Both total Cu and As were highly accumulated in the seedlings, and their accumulations were influenced by treatment ($p < 0.01$). Concentrations of Cu in the shoot and root were linearly correlated with the Cu concentration in the sand ($R^2 = 0.756$ and 0.948, respectively, both $p < 0.001$). However, the relationship between Cu concentrations in the shoot and root with the Cu concentration in the sand deviated from linearity with the addition of As in the sand ($R^2 = 0.890$ and 0.921, respectively, $p < 0.001$). A peak of Cu concentration in the shoot was observed with the addition of As, while a plateau of Cu concentration was reached in the root. Moreover, As concentration in seedlings had a slightly negative correlation with Cu concentration in the sand ($R^2 = 0.275$ and 0.100, $p < 0.05$ and $p = 0.08$ for shoot and root, respectively).

Improving the Environmental Assessment of Complex Composition Substances and Mixtures for Chemicals Management

RP032 Bioaccumulation of pine oil in fish

C.L. Chen, Stockholm University / Environmental Science and Analytical Chemistry; M. MacLeod, ITM - Stockholm University / Environmental Science and Analytical Chemistry; M.S. McLachlan, Stockholm University / Environmental Science and Analytical Chemistry (ACES)

Assessing the bioaccumulation of substances with unknown or variable composition like essential oils is required by the REACH regulation but problematic. Our previous study with model compounds has demonstrated that it is feasible to determine the bioconcentration factor of complex mixtures through a dietary exposure. Herein we present an in vivo bioaccumulation study of one real essential oil – pine oil – by dosing it and several benchmark chemicals to rainbow trout. After feeding one contaminated meal (0.7% of body weight), the fish were fed with clean food daily. Five fish were sacrificed after 8 h, 1, 2, 4, 7, 14, 21 and 28 days. The gastrointestinal tract (GIT) and the rest of the fish body were analysed separately for the target chemicals (currently undergoing). The data will be mined to derive the depuration rate constants (overall k_T and growth corrected k_2). These will be used to calculate the kinetic bioconcentration factor (BCF) of the target chemicals. The bioaccumulation parameters (k_T, k_2 and BCF) for the non-targeted minor components in the pine oil will also be explored by comparing their relative peak areas in the chromatograms. As a case study, this will, for the first time, demonstrate how we can assess the bioaccumulation of natural complex substances such as essential oils.

RP033 IFRA environmental standards and RIFM program advances update for 2017

A. Lapczynski, D.T. Salvito, Research Institute for Fragrance Materials (RIFM) / Environmental Science; M. Vey, International Fragrance Associations; C. Gonzalez, IFRA

To assure safety of fragrance ingredients in consumer products, International Fragrance Association expanded the fragrance industry's self-regulatory safety program with the development of IFRA Environmental Standards for both risk and hazard in 2008. Fragrance material risk assessments for these Standards are incorporated in the Research Institute for Fragrance Materials' (RIFM) testing program in coordination with its Expert Panel. To identify materials for risk assessment refinement, fragrance materials were screened using the RIFM Environmental framework and 2008 IFRA volume of use survey as reported for both Europe and North America. The Framework for this evaluation was published in Environment Toxicology and Chemistry (Salvito et al., 2002, 1301-1308). In addition, hazard assessment on these materials was also performed and reviewed. As a result nearly 3,000 materials were screened with preliminary risk quotients estimated to rank priority materials for risk assessment refinement. In an effort to provide greater transparency to the IFRA Environmental Standards, RIFM reports the most recent results of these additional tests (for both risk and hazard assessments) at both the annual SETAC NA and Europe meetings. These studies include persistence testing (ready biodegradation tests and die-away studies), bioaccumulation, and acute and chronic aquatic toxicity. Incorporating these new data in a second tier risk and hazard assessment for these materials will also be presented.

Incorporating Environmental Toxicology Into the Classroom

RP034 Use of closed ecological systems in an introductory biology class as a way to investigate the ramifications of contaminants on ecosystems

S.M. Weir, Queens University of Charlotte / Biology

There has been an increased interest in embedding authentic research experiences into undergraduate biology courses. This method of "learn by doing" or "apprenticeship" model promotes the idea that students learn best by actually participating in the scholarly activity of a particular field. There are few undergraduate toxicology programs in the country and most students entering undergraduate biology programs are probably not focused on a career in environmental toxicology. Exposing students to stimulating, authentic, toxicology experiments as undergraduates can be extremely beneficial to introduce students to the field. I describe a simple experiment that I have used in the ecological/evolutionary portion of the introductory biology courses for majors. The project uses "closed ecological systems" to serve the dual purpose of getting students to consider the ecological topics covered in lecture and providing the opportunity to investigate toxicological principles. Students are given a simple system of algae (*Selenastrum sp*) and *Daphnia magna* in sealed 125 mL jars. A microbial community that lives within in the algae and *Daphnia* cultures are present but not specifically quantified. The system is set up in moderately hard water and fertilizer ($(NH_4)_2SO_4$ and K_2HPO_4) is added to provide nutrients. The systems then resemble microcosms of Earth in that they are essentially "closed" except to light energy. There are four treatments each with six replicate jars: (1) Control with algae only, (2) Control with algae and *Daphnia* (controls are shared across groups), (3) Treatment with algae only, (4) Treatment with algae and *Daphnia*. Students work in groups to decide how to "disturb" their system. I allow students to come up with their own treatments, but I suggest simple ideas: altering fertilizer levels, adding an herbicide, adding an insecticide, altering thermal regimes. Over the course of several weeks, students monitor algal density (how green the jar is) and daphnia numbers. At the end of the experiment (*Daphnia* often die out around week 3 or 4), students learn to manipulate data and create figures summarizing results. They then present the data to the class. Student response to the project has been very positive, providing further evidence that students prefer "authentic" research experiences. Future goals include quantifying students learning outcomes as well as student self-assessment before and after the experiment has run.

RP035 Teaching Environmental Toxicology through an Applied and Interactive Risk Assessment Course

A. Heaton, University of South Alabama / Biology; L. Lovett, E. Miller, University of South Alabama / Environmental Toxicology Program; S. Glaberman, University of South Alabama / Biology

Ecotoxicology is by nature an applied discipline. Therefore student motivation and career prospects are directly related to the ability to address real-life environmental challenges using the latest available tools. Our course utilized a semester-long risk assessment focusing on three insecticides (carbofuran, endosulfan, and imidacloprid) with different modes of action to walk students through the foundations of ecotoxicology. Every other week, students were exposed to the latest analytical methods and tools covering different subject units, including regulatory history, chemical mode of action, environmental fate and transport, laboratory toxicity studies, and field studies, and applied these approaches to their case-study chemicals. At the end of the semester, students delivered final oral integrated summaries of their chemicals. One success of this course is that students actively learned about ecotoxicology and were ready to apply these tools more broadly. One challenge was the difficulty in giving students enough practical knowledge to interpret the meaning of their analyses.

RP036 Adventures with Paracelsus: Increasing toxicological literacy among rising high school seniors enrolled in SSTP at the University of Florida

A. Buerger, University of Florida / Environmental and Global Health; F. Lambert, University of Florida / Center for Environmental and Human Toxicology; S. Humes, A. Wormington, University of Florida / Environmental and Global Health; J. Nicholas, University of Florida / Physiological Science; D. Faulkner, University of California Berkeley; M. Koroly, University of Florida / Biochemistry and Molecular Biology; J.H. Bisesi, University of Florida / Environmental and Global Health

Scientific outreach remains one of the most difficult, yet fundamentally important aspects of a scientist's job. Encouraging scientific endeavors and enthusiasm among young audiences remains challenging, yet vital as the next generation of scientists enter college. Through the Student Science Training Program (SSTP), a summer research apprenticeship program at the University of Florida (UF), rising high school seniors in summers 2016 and 2017 were introduced, most for the first time, to concepts in environmental science and toxicology during the course of a seven week class taught by graduate students working at the Center for Environmental and Human Toxicology. This interactive course taught the fundamentals of the field through various case studies and in-class activities. Based on student and teacher feedback, the course was altered to be more interactive, especially for topics of greater difficulty, and to use different case studies the second year that were better illustrative of toxicological concepts. Communicating these ideas to the high school students was challenging, enjoyable and rewarding. Main successes were observed when the course was made relatable to the students through the use of real world examples, and the development of course mascots to foster a sense of enthusiasm among students about the material. Challenges included limiting the curriculum to information that was vital for a basic understanding of toxicology, but still comprehensible to our audience during the accelerated course timeline. This presentation will highlight our dynamic approach to develop an environmental toxicology course material for young audiences, the successes and challenges faced by the UF graduate students along the way, and the importance of reviewing the effectiveness of course materials and refining the curriculum as needed. Our goal is to showcase potential effective learning methods for improving science course curriculum and in doing so not only to assist but to inspire other scientists in educational outreach among younger audiences.

Microplastics in the Aquatic Environment – Fate and Effects

RP037 New techniques to qualify and quantify fine microplastics in surface water

Y. Kameda, Chiba Institute of Technology / Creative Engineering

The occurrence of Microplastics (MPs) is of great concern in aquatic environment, especially ocean. Many current studies evaluate MPs ranged from 100 µm to 5 mm. However, MPs used in personal care products and other industrial processes are reported to be smaller according to previous reports. Because MPs are very important substances to current economic activities, new materials for MPs will be needed such as cellulose. But before the alternative of the materials, contamination levels of these fine MPs in aquatic environment should be revealed.This study tries to apply new techniques to qualify and quantify these MPs including "ordinary" MPs. One technique is Infrared microspectroscopic method. Infrared microspectroscopic method is very useful to qualify less than 100 µm MPs. Moreover some spectrometers are equipped with mapping system. This system enables automated multi-point mapping, line mapping and IR Imaging analyses of a microscopic area with a manual sample stage and a single element detector. The microscope system automatically scans the specified points or area, rapidly collecting a full spectrum of each point without moving the sample stage. It can provide a measurement of a maximum area of 400 µm square. The combination of mapping system with the automated XYZ stage can provide a wide area analysis capability. The other technique is TOC analysis. Some TOC analyzers can measure particulate plastics. Though TOC analyzer can not identify plastic compositions, it can be useful to quantify and evaluate total mass of MPs. This presentation introduces qualification and quantification of MPs extracted from river surface water, seawater and sand beaches by above two techniques.

RP038 Degradation of Biodegradable Plastics in a Salt Marsh Habitat

J.L. Dekle, The Citadel / Biology; R. Leads, College of Charleston / Biology; J.E. Weinstein, The Citadel / Biology

Plastic debris is ubiquitous in the environment and is considered to be a significant anthropogenic threat to ecosystem health. In Charleston Harbor, an estimated 7.5 tons of plastic debris litter the shoreline. Once in the environment, plastic debris may fragment into microplastic particles (< 5 mm) due to UV light exposure, physical abrasion, and biological degradation. These factors are particularly evident in estuaries, increasing the potential for fragmentation and microplastic accumulation. The accumulation of plastic debris has created a demand for biodegradable alternatives to single-use plastic items such as plates, cups, and grocery bags. To meet ASTM standards, biodegradable plastics must exhibit 60-90% decomposition within 60-180 days in a composting environment. The objective of the present study was to investigate the degradability of biodegradable polymers in a salt marsh estuary and to compare the degradation patterns of biodegradable polymers to synthetic polymers. Strips (15.2 x 2.5 cm) of 0.51 mil high density polyethylene (HDPE), 0.5 mil biodegradable high density polyethylene (BioHDPE), 0.64 mil Mater-Bi® biodegradable polylactide (BioPLA), Ingeo™ compostable polylactide (cPLA), polyethylene terephthalate (PET), polystyrene (PS), and biodegradable polystyrene (BioPS) were field-deployed in May 2017. Subsamples of strips were collected at 4, 8, 16, and 32 weeks and were monitored for fragmentation, UV transmittance, and changes in surface area and weight. Previous research investigating the degradation of plastic debris in salt marshes reported microscopic surface erosion and surface delamination in as little as 8 weeks, leading to the release of microplastic fragments and fibers in the early stages of degradation. The present study hypothesized that biodegradable plastics will exhibit surface delamination more quickly than synthetic plastics, further contributing to microplastic accumulation in the salt marsh environment. These results will provide an understanding of the rate of degradation of biodegradable plastics in the salt marsh as well as the degree to which biodegradable plastics produce microplastic fragments compared to that of synthetic plastics.

RP039 Sorption of Persistent Organic Pollutants to Microplastics in the Marine Environment

L. Mai, Jinan University / Environmental Science; L. Bao, C. Lu, Jinan University; E.Y. Zeng, Jinan University / School of Environment

Microplastic (MP) contamination in the marine environment has drawn increasing global attentions. Because MPs may carry and transport persistent organic pollutants (POPs), understanding the sorption behavior of POPs on MPs in sea water is of great importance. Sea surface water of the Bohai and Huanghai Seas offshore China were sampled for MPs at the micro-size scales (100 μm–5 mm) to evaluate in situ dissolved-microplastic phase partitioning of various classes of POPs, including polycyclic aromatic hydrocarbons (PAHs), dichlorodiphenyltrichloroethane (DDTs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs). Sorption depended on the type of MP (e.g., polypropylene (PP), polystyrene (PS) and polyethylene (PE)), which are potential markers for delineating sources and mechanism of transport of POPs on MPs. In the laboratory, we obtained the K-values for water-microplastic distribution coefficients (mostly $\log K > 5$), which should be consistent with sorption of POPs to microplastics observed in the field. Preliminary results indicate that POPs with primarily terrestrial sources to the marginal seas offshore China are co-transported through sorption to MPs released from anthropogenic sources. The current study provides insight into the total co-mingled pollution of MPs and POPs in the marine environment worldwide. The contribution of MPs to the entire input of POPs to the global ocean, and the subsequent biological risk assessment of this combined pollution, should be further assessed.

RP040 Identifying and quantifying microplastics in the effluent of advanced wastewater treatment systems

S. Fortin, Virginia Institute of Marine Science / Biological Sciences; B. Song, Virginia Institute of Marine Science, College of William and Mary / Biological Sciences; C. Burbage, HRSD

Microplastics (plastic pieces < 5mm) are an emerging contaminant in aquatic environments. They are capable of sorbing persistent chemical contaminants and being ingested by numerous organisms with consequences including changes in energy allocation and decreases in reproduction. Identifying the sources of microplastic contamination is necessary in order to evaluate ecological risks of microplastic contamination and for better management practices. Wastewater treatment plants (WWTPs) have been shown to be a significant source of microplastics to the aquatic environment. Microplastics in WWTP effluent were previously identified and quantified, but few studies have examined the removal efficiency of microplastics in advanced treatment systems, such as nano-reverse osmosis filtration and activated carbon filtration. In addition, the polymer types of microplastics discharged from the advanced treatment processes have not been identified. To fill this knowledge gap, microplastics in WWTP effluent from different advanced treatments need to be quantified and the polymer types determined. This study examined effluent water from a WWTP with standard tertiary treatment, as well as effluent from nano-reverse osmosis and activated carbon filtration systems. A new method for isolating, quantifying, and determining the polymer type of microplastics from the effluents was developed. Briefly, microplastic particle samples from the above described WWTP effluents were concentrated and prepared for particle analysis. After the completion of sample preparation, Raman microspectroscopy and a polymer database (St. Japan) were used to identify and quantify various types of microplastic particles. Both fragments and fibers were found in the effluent of a tertiary WWTP, as well as in the effluent of the advanced nano-reverse osmosis and activated carbon filtration systems. Further analysis will be conducted to identify microplastic types and evaluate removal efficiency of microplastics by the two advanced treatment systems.

RP041 Considering the relative hazard of microplastics and total suspended solids in WWTP effluent and receiving waters

K.A. Connors, C. Eylem, S.E. Belanger, S.D. Dyer, The Procter & Gamble Company / Environmental Stewardship and Sustainability Organization

Microplastics have been observed and quantified in a variety of environmental media, including in waste water treatment plant (WWTP) effluent. These small, insoluble polymeric particles have been implicated by scientists as having potential for impacts in freshwater and marine pelagic and sediment environments. Microplastics are thought to produce biological effects through direct physical mechanisms, and not due to intrinsic toxicity. These physical mechanisms may be similar to the effects seen by microparticles, such as clays, silts, and/or other particles that comprise total suspended solids (TSS). Here, we contrast the concentration and risk associated with microplastic release from WWTP to permitted and measured TSS. Concentrations of solids permitted for WWTPs was investigated by mining the ICIS-NPDES Permit Limit database. Distributions of solids levels allowed varied by treatment type and by geography (e.g., state). USEPA's STORET database was also mined to assess the central tendencies of sediment solids levels in receiving waters within the USA. This too was dependent upon geography, due to climate and geological attributes. Overlaying the wastewater treatment plant data onto the receiving water data provides an overview of when WWTPs solids are less than, equal to, or more than the receiving water content. This overall context provides a set of realism that is not currently accounted for at large geographical scales when discussing the discharge of microplastics from WWTPs. Via iSTREEM Version 2, we modeled microplastics loads to determine the proportion of solids due to microplastics. Lastly, we compared species sensitivity distributions for suspended solids to these exposure metrics to determine the risks associated with suspended solids and the proportion potentially attributed to microplastics. This is the first paper, we are aware of, where risks from microplastics and solids have been holistically assessed at such a large geographical scale.

RP042 Microplastic Pollution in an Urban River in Western Canada

T. Bujaczek, MacEwan University / Physical Sciences; D. Locky, MacEwan University / Biological Sciences; M. Ross, MacEwan University / Physical Sciences

Research focused on microplastic pollution in the freshwater environment is beginning to take form to understand potential sources and sinks of this widespread pollutant. This project focuses on investigating microplastic occurrences and potential point sources within the urbanized North Saskatchewan River in Edmonton, Alberta, Canada. Water samples were collected with 53µm plankton nets at 10 sites along this river, both upstream and downstream from a likely effluent source (i.e., wastewater treatment plant). Following sampling, wet peroxide oxidation and density flotation were included in the procedure to eliminate organic material and separate the microplastics from sediments. Preliminary analysis using a dissecting microscope revealed an assortment of microplastic fibres, fragments, films, and beads with varying size and colour characteristics. Microplastic fibres constituted a significant proportion of microplastic abundances in preliminary water analysis. The chemical identities of the microplastic polymers will be confirmed using Raman microspectrophotometry to determine differences in abundances amongst fiber types. This study strives to understand the significance of urban populations on microplastic contamination for urban rivers in Western Canada, and the generated occurrence data will provide a valuable baseline for future monitoring studies.

RP043 Insights into sources, presence and possible effects of microplastics in agricultural soils

A. Palmqvist, I.A. Larsen, Roskilde University / Science and Environment; S.L. Vestergaard, J.A. Madsen, EnviDan A/S; J. Magid, University of Copenhagen / Plant and Environmental Sciences

While plastic pollution of the aquatic environment has received large scientific attention during the last two decades, and more recently also been subject to intense public and political debate, it is only very recently that the potential problem of plastic pollution of terrestrial environments has been raised in scientific, regulatory and political communities. Current knowledge on the presence and possible effects of plastic litter and in particular of microplastic (MP) in soils is thus very limited making it impossible to assess if release of MP to the terrestrial environment is causing environmental harm. Along with seashores, agricultural soils, due to intensive manipulation, are likely to be among the terrestrial environments receiving the largest input of MP from multiple sources. In addition to littering, use of non-woven and mulching fabrics, and general management practices, MP may be released to agricultural soils through addition of different types of organic fertilizers, such as sludge from wastewater treatment (WWT) and composted household waste. We will present the main conclusions from two independent studies on MP presence in: 1) various fractions from a WWT plant including sludge ready to use for agricultural purposes, and 2) soils that have received organic fertilizers in the form of sludge, compost and cow manure over a 15 year period. The former study showed that the WWT plant was quite efficient in retaining MP, with the majority of the MP ending up in the sludge. However, it also showed through which processes MP is currently removed from the plant during the treatment, and a potential for further optimization in MP removal from the sludge with existing WWT processes and technologies was indicated. The study on soils fertilized with different organic resources confirmed that using sludge and compost for soil amendment is likely to increase the amount of MP compared to unfertilized soils, when these resources are used over many years. However, it is important to keep in mind that recycling of organic resources is an important means of preserving phosphorus and other plant nutrients. Since it is inevitable that agricultural soils is polluted with MP from different sources, it is thus important to understand the possible effects on important soil dwelling organisms. Therefore, we will conclude the presentation by showing results from a few preliminary studies on MP effects on earthworms that were carried out in one of our laboratories.

RP044 Are There Microbeads in Sediment, Dreissenid Mussels, and Anurans of the Littoral Zone of the Upper St. Lawrence River?

M. Schessl, C. Johns, S. Ashpole, St. Lawrence University / Environmental Studies;

Global plastic production now exceeds 260 million tons per year. Much plastic waste ends up in marine environments. In both marine and freshwater environments, larger plastics abrade or photo-degrade resulting in persistent micro-particles. The ecological effects of micro-plastics in the marine environment are still incompletely understood; but, far less attention has been paid, until recently, to freshwater systems. In an effort to assess the extent that micro-plastic beads may have infiltrated the freshwater ecosystem of the upper St. Lawrence River, four sites were sampled along 100 km of shoreline, from Alexandria Bay to Waddington, NY. Twelve sediment samples and 114 Dreissenid mussels were collected from the littoral zones. And, forty-one road-killed anuran amphibian specimens were collected adjacent to the river. Of the four sediment sampling sites, two were found to contain microbeads in subsamples after sieving using a 500 micron mesh. No microbeads were detected within any of the Dreissenid mussel or anuran digestive tracks. The mussels we collected were likely too small to ingest microbeads greater than 35 microns; a bottom feeding species of fish such as the Round Goby (*Neogobius melanostomus*) may be a viable monitoring species. Analysis of treated effluent of wastewater treatment plants as point sources of micro-beads/plastics is recommended to ascertain inputs for possible transfer to lower trophic levels of the St. Lawrence River ecosystem.

RP045 Morphometric Effects of Microplastics on Larval Zebrafish

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Microplastics are plastic particles less than 5mm in diameter as defined by National Oceanic and Atmospheric Administration (NOAA). Microplastics are materials that are formed from synthetic or semi-synthetic organic compounds or the degradation of larger plastics. These particles can vary with composition by the addition of plasticizers, i.e. chemical additives that increase the plasticity or viscosity of plastics. Plasticizers are not bound to the polymer matrix of plastics, which leaves them soluble and susceptible to migration. Additionally, ingestion of microplastics by various marine species has been documented and is of growing concern. Both microplastics and plasticizers are now ubiquitous in waterways, beaches, sediments and biota across the world. The aim of this study is to determine whether there are gross morphological variations in sac fry Zebrafish as a result of exposure to weathered and pure microplastics. Embryos were exposed at 3 hours post fertilization (hpf) to 96 hpf with samples of weathered microplastics from Newark Bay, as well as pure plastics at concentrations of 1 µg/mL and 10 µg/mL. No observations of gross lesions or altered development were noted during daily observations over a 5 day period. The Newark Bay microplastics were analyzed via pyrolysis GC-MS to determine composition. It was determined that the three analyzed samples were composed of polyethylene, polypropylene, and PVC and vinyl acrylate. Morphometric data were analyzed via SigmaPlot and no significant changes were noted between the controls and the treated groups in the embryonic Zebrafish samples for the Newark Bay weathered samples. Significant changes were seen in total body length in the polyethylene 10 µg/mL exposure. Significant changes were seen in pericardial sack size in polyethylene low density (LD) 10 μg/mL, polyethlene high density (HD) 1 μg/mL and 10 μg/mL, and polystyrene 1 μg/mL and 10 μg/mL exposures of pure plastics samples.

RP046 Biological activities of extracts from North Pacific Gyre Plastics with UV-treated and untreated materials using in vitro and in vivo models

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Plastic debris is an emerging worldwide threat to marine biota. Plastic samples recovered from the North Pacific Gyre (NPG) along with UV-irradiated virgin plastic (UVP) and non-irradiated virgin plastic (VP) resided in saltwater for 30 days under sunlight. Following solid phase extraction, samples were fractionated with sequential methanol elution from 10-100% and evaluated using in vitro assays assessing estrogen receptor (ER) and aryl hydrocarbon receptor (AhR) activities. Extracts were also evaluated using juvenile fish to evaluate ER (vitellogenin-VTG) and AhR (Cytochrome P450 1A-CYP1A). Estradiol equivalence (EEQ) values were localized in the 70% methanol fraction and were 5.9, 9.1 pg/L and 0.1, for NPG, UVP and VP, respectively. TEQ analysis for AhR indicated highest activity in the 90% methanol elution with greater activity in NPG than in VP or and UVP (TEQ= 917 ng/L, ND and ND, respectively). After Larval medaka (3 dph) were exposed to 0.01% methanol plastic extracts for 5 days, CYP1A mRNA was induced 28.9-fold in plastic obtained from the NPG relative to controls ($p=0.011$; ANOVA Dunnett's test). However, UVP and VP showed no significant differences. Analytical chemistry analyses indicated TEQ concentrations of co-planar PCBs of 337 ng/L in the 90% fraction of NPG plastics, and < 1.0 ng/L in all other fractions and plastic types. VTG induction was observed in the UVP (6.9-fold, $p=0.012$; ANOVA, Dunnett's test). These results indicated that weathered plastic from the Pacific Gyre likely had adsorbed ligands for AhR and that UV treatment of plastics may release ER agonists that produce in vivo responses in fish.

RP047 Evaluating chronic terrestrial toxicity of a solid polymer

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Over the last several years there have been a range of articles reporting the presence of microplastics in rivers, lakes, and oceans. Although the major sources for input of these particles into waterways are breakdown of larger plastic objects and dust from tires, it has been suggested that the ability of these micron and sub-micron particles to pass through wastewater treatment plants (WWTPs) may contribute towards microplastic's input into the environment. However, more recent publications have reported removal efficiencies of these particles routinely range from 90-99% in properly operating WWTPs. In a previous study we reported the removal of two solid cross linked styrene acrylate copolymers from a waste stream was primarily through sorption to sludge and subsequent settling. After treatment, sludge could be incinerated, sent to landfill, or reapplied to farm land as fertilizer. Consequently, it is very important to understand the potential toxicity of these particles to terrestrial organisms. In this study, we evaluated the chronic terrestrial toxicity of a solid cross linked styrene acrylate copolymer according to procedures in the Organization for Economic Cooperation and Development (OECD) Guideline 222 (Earthworm Reproduction Test). Adult earthworms (*Eisenia fetida*) were exposed to the solid polymer at five concentrations: 12.3, 37.0, 111, 333 and 1000 mg/kg dry artificial soil for 28 days at 20 ± 2°C with a controlled light - dark cycle of 16 hours light (400-800 lux) and 8 hours dark. After 28 days, adult earthworms were removed to evaluate mortality and growth. The cocoons and the soil were returned to the test chambers and the exposure continued for another 28 days to evaluate effects upon reproductive output. Each treatment group had 4 replicated test chambers and the control group had 8 replicated test chambers, with each replicate containing 10 earthworms. Preliminary results show that there was no mortality at any test concentration level at Day 28. Furthermore, there was no statistically significant difference from the control at any test level on the body weight of the adult earthworms. At Day 28, cocoons were also observed in all replicates for each control and test concentration. Final results will be presented and discussed at the conference.

RP048 Bioaccumulation and ecotoxicity of microplastics at environmentally relevant concentrations to the blue mussel Mytilus edulis

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In the second half of the 20th century, the production of plastic expanded resulting in the elaboration of various products from diverse plastic types. Nowadays, plastic products can be found in several fields (mainly as packaging), such as health, energy generation, construction, automotive industries, electronics, and textiles. Since the early 1970s, the presence of floating plastic has been reported in every marine water with great accumulation in the Northern Hemisphere subtropical gyres in theNorth Atlantic(e.g Desforges et al., 2014). In recent years, the presence of plastic debris called microplastics (MPs) which are the result of macroplastic's fragmentation has also been reported in aquatic ecosystems even in the most remote places such as the Artic (Cozar et al., 2014). Several studies have reported the presence of MPs in marine organisms but it appears necessary to investigate their potential toxicity especially at environmentally relevant concentrations. The aim of our study is to evaluate the putative bioaccumulation and ecotoxicity of polypropylene and polyethylene MPs towards blue mussel *Mytilus edulis* from the Region of Pays de La Loire (France). Mussels were exposed in the laboratory during 10 d at two environmentally relevant concentrations of 8ng/L and 10μg/L (Desforges et al, 2014; Collignon et al. 2012), but also to a much higher concentration (100 μg/L). The exposure was follow up by 10 days of depuration (in clean water without any MPs). MPs fragments were prepared in the laboratory from commercially available product and selected according to a previous study conducted in situ in the region of Pays de la Loire (Phuong et al. 2017). Analysis of MPs in organisms and biodeposits (feces and pseudofeces) after digestion, filtration and observation in microscopy coupled with infrared spectroscopy will be presented. Concerning potential toxicity effects, feeding rate, potential detoxification and oxidative stress mechanisms through measurement of enzymatic activities of Glutathione-d-transferase (GST), Catalase (CAT) and superoxide dismutase (SOD) will be presented as well as parameters of the immune system and DNA damage.

RP049 The effect of a microplastic-associated legacy pollutant on the feeding preferences of microzooplankton and their predators

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Microplastics are becoming more abundant in estuarine systems due to increased production of plastic products and proximity to land based sources. These particles (< 5mm) have been demonstrated to be directly ingested by zooplankton, benthic invertebrates, shorebirds and fish species. Marine organisms can also be exposed to microplastics via their prey. Furthermore, the surface of plastic attracts harmful, lipophillic compounds, such as the legacy pollutant dichlorophenyltrichloroethane (DDT), which can leach into the tissues of marine organisms upon ingestion. This study used larval inland silversides, *Menidia beryllina*, as predators, and tintinnid ciliates, *Favella* spp., as microzooplanton prey, to quantify plastic ingestion via different routes. Polyethylene microspheres (10-20µm) treated with DDT and virgin microspheres were used to determine whether the presence of plastic-associated pollutants affects the feeding preference of larval fish and their prey. We hypothesized that larval silversides feed differentially on prey exposed to DDT-laden microplastics due to the potential effects of DDT on prey predator-avoidance behavior and that trophic transfer is a more important route for microplastic ingestion. After a two-hour feeding period, trophic transfer treatment groups ingested a significantly higher number of microplastics than direct ingestion treatment groups, suggesting ingestion of contaminated prey could be an important route for microplastic exposure. Larvae also ingested significantly more prey exposed to DDT-laden plastics than prey exposed to virgin plastics. DDT seems to play a role in the prey preference of larval fish, and it is possible that the chemical affects the predator avoidance behavior of *Favella*. Growth parameters were also measured 30 days after a 2-hour microsphere feeding session to determine the effects of microplastic ingestion on larval growth. We hypothesize that larvae exposed to microplastics will experience slower growth rates, likely due to the nutrient-diluting effects of plastic ingestion. Microplastic gut retention time of larval silversides was also determined. Our findings demonstrate that the trophic transfer of micro plastics likely originates at the base of estuarine food webs and that organisms cannot differentiate between virgin and contaminated microspheres. This is concerning given that the inland silverside and *Favella* microzooplankton are common prey items for commercially important fish species.

RP050 Toxicicty of nanopolystyrene plastics and their interactions with polycyclic aromatic hydrocarbons on early life stages of zebrafish

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Plastics are the major components of marine debris, and recent studies have suggested their threat to both marine and freshwater life. Plastics debris can be found at the macro, micro and nanoscale, are typically persistent in the environment, and can be fragmented by weathering into smaller and numerous particles. This project investigated the distribution and toxicity of nanopolystyrene beads (NanoPS, 42 nm diameter) to early life stages zebrafish. It was also investigated the interactions of a complex mixture of PAHs (Elizabeth River Sediment Extract – ERSE) to the NanoPS toxicity. Zebrafish embryos at 6 hpf were dosed with 0.1, 1 and 10 ppm NanoPS, and all assays were performed at 96 hpf. NanoPS did not induce mortality in any of the tested concentrations, though developmental deformities such as bent notochord and fin deformities were evident at 1 and 10 ppm. Plastic uptake and distribution were assessed using green fluorescent NanoPS, which was found predominantly in the gastrointestinal tract, liver, gall bladder, pancreas, as well as in the heart and brain. Further analyses of protein thiol oxidation and cellular redox state indicated signs of oxidative stress at 10 ppm NanoPS. Single exposure to a complex mixture of PAHs such as ERSE (0.1%, 0.5%, 1%, 2% and 5%) induced expected cardiac deformities such as string heart and pericardial edema, as well as bent notochord and jaw and fin deformities, all with higher rates at 2% and 5% ERSE. PAHs exposure also caused increased EROD activity at 1%, 2% and 5% ERSE. Interestingly, 10 ppm NanoPS co-exposure with 1%, 2% or 5% ERSE reduced the effects of the previously established NanoPS toxic effects. Decreased developmental abnormalities were detected in all the co-exposure groups. Such reduced rates were greater for string heart, pericardial edema and jaw deformities. In addition, the NanoPS co-exposure with 1% or 2% ERSE also blocked increased CYP1A activity, elicited by ERSE alone exposure. Taken together, our data point to a possible role of oxidative stress on the toxicity of NanoPS during zebrafish early development possibly associated with mitochondrial dysfunction, as already suggested with other plastic particles. A striking effect of NanoPS was related to decreasing PAHs toxicity. As organic pollutants are known to absorb to plastics, it is possible that NanoPS are acting as a target for PAHs absorption, decreasing their concentration in the exposure medium and therefore their toxicity.

Pharmaceuticals in the Environment – Potential Environmental and Human Health Impacts

RP051 Transformations of Pharmaceutical Compounds in Wastewater Effluent Post-UV and ClO- Disinfection

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Over the past two years, a team of chemists and biologists have studied the effect of newly installed disinfection systems in wastewater treatment at the O'Brien and Calumet plants on reducing the concentrations of estrogenic, androgenic and neuro-endocrine pharmaceutical compounds. Both plants are 250 MGD in size operated by the Metropolitan Water Reclamation District of Greater Chicago. The Calumet facility installed chlorination/dechlorination whereas the O'Brien facility uses UV disinfection, making it the largest wastewater treatment plant using UV disinfection in the world. It was hypothesized that disinfection processes would lead to the transformation of pharmaceutical compounds. Concentrations of antidepressant pharmaceuticals and estrogenic compounds were measured in effluent samples pre- and post-disinfection across a 12-month period of operation. Compounds tested included the full suite of anti-depressants, plus carbamazepine, estradiol, estrone, and nonylphenols. Reductions in the concentration most compounds were observed. Bench-scale experiments measuring the transformation of 13 individual pharmaceuticals in deionized water and wastewater by either UV or chlorination treatment were performed. These experiments were designed to mimic the process used at scale and assess each compound's sensitivity to the disinfection process. HPLC-MS was used to measure the concentration of each compound before and after treatment while also detecting transformation products. Overall, the reaction processes with chlorination are complex leading to a variety of reversible and irreversible by-products, often involving chloramine formation. UV treatment transforms a wide range of compounds with products generally undetectable by HPLC-MS. Agonistic activity of the antidepressant pharmaceuticals (pre- and post-UV treatment) was evaluated using cell-based receptor assays (estrogen, progesterone, androgen). Overall, the in vitro chemical and bioassay data indicate that disinfection processes do transform a majority of the pharmaceuticals reducing overall loads and producing a complex set of by-products which initial testing indicates may not have estrogenic, androgenic or progestogenic activity. These results are also being correlated to health and reproductive measurements of free and caged fish populations living upstream and downstream of effluent discharge and fish exposed to effluent pre- and post-disinfection.

RP052 Groundwater and surface water exposure assessments of a veterinary drug (Revalor-XR) containing trenbolone acetate and 17β-estradiol

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The use of veterinary drugs has led to increasing interest in the fate of these compounds in the environment. Revalor-XR is an extended-release implant in steers and heifers that contains trenbolone acetate and 17β-estradiol (17β -E2) as active pharmaceutical ingredients (APIs). Both APIs are metabolized in situ resulting in the excretion of 17β-trenbolone

(17β-TB), 17α-trenbolone (17α-TB), trendione (TBO), 17β -E2, 17α-estradiol (17α-E2), and estrone (E1). The environmental exposure assessment of Revalor-XR was performed on these six compounds. Data on environmental fate and behavior of these metabolites were collected from various laboratory studies. They were then aggregated to generate representative values for surrogate compounds to represent the trenbolone compounds and the estradiol compounds. The surrogate compounds were used to assess potential exposure at the feedlot and field scale using USEPA's EXPRESS, SCIGROW and other models The exposure assessments at the feedlot and field scale involve many overly conservative assumptions for calculating exposure concentrations; however, they were useful for focusing further efforts on the most significant exposure pathways and eliminating minor pathways. The major exposure pathways were then evaluated on an aggregate basis in a watershed scale exposure assessment. This was performed based on the geographical characteristics of representative watersheds in Texas and in Iowa, using USEPA's BASINS model. The outputs of the watershed-scale modeling were expressed as the upper 90th percentile of the monthly maximum 21-day moving averages for the surrogate trenbolone compound and the surrogate estradiol compound; these values were used in the risk assessment. These three scales of exposure assessments demonstrated that potential environmental exposures from Revalor-XR can be significantly overestimated when only the estimated environmental concentrations from the feedlot- and field-scales were used.

RP053 Evaluating iodide recycling inhibition as a novel molecular initiating event for thyroid axis disruption

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The enzyme iodotyrosine deiodinase (dehalogenase, IYD) catalyzes iodide recycling and promotes iodide retention in thyroid follicular cells. Loss of function or chemical inhibition of IYD reduces available iodide for thyroid hormone synthesis, which leads to hormone insufficiency in tissues and subsequent negative developmental consequences. Iodide recycling by IYD is especially critical under conditions of low dietary iodine and in low iodine environments, including most freshwater ecosystems. We evaluated the impact of IYD inhibition on the thyroid axis of the model amphibian species *Xenopus laevis.* Tadpoles were exposed to 3-nitro-L-tyrosine (MNT, a known IYD inhibitor) from premetamorphosis (NF stage 51) to metamorphic climax (NF stage 61-62) in 30-day static renewal waterborne exposure experiments. Under a low iodine diet, exposure to 7.4, 22.2, 66.7, and 200 mg/L MNT was tested with an additional treatment of 200 mg/L MNT with iodide supplementation. IYD inhibition was also tested with three separate diets with different iodine content to exposure of 50, 100, 200, and 400 mg/L MNT. Exposure resulted in markedly delayed metamorphosis and glandular hypertrophy. In tadpoles exposed to the highest concentrations (200 and 400 mg/L), development was completely arrested at NF 58-60, a stage when demand for thyroid hormone dramatically increases. IYD inhibition led to reductions in thyroxine (T4), increased monoiodotyrosine (MIT), and increased diiodotyrosine (DIT) concentrations in the plasma. The dose-response for these effects was dependent on dietary iodine content. Reduced T4, increased MIT and DIT, and thyroid gland compensatory response (sodium/iodide symporter gene expression) were found across the range of concentrations tested, even in treatments where tadpoles reached metamorphic climax. Supplementation with iodide negated the effect of IYD inhibition on metamorphosis and plasma T4, effectively 'rescuing' the exposed tadpoles. In addition, MIT and DIT in the plasma were greatly increased, demonstrating inhibition of iodide recycling. These results establish toxicological relevance of IYD inhibition and validate this novel molecular initiating event in amphibians. Given the highly conserved nature of the IYD protein sequence, IYD inhibition may be worth further investigation as a molecular initiating event for thyroid axis disruption with cross-species relevance. This abstract does not necessarily reflect USEPA policy.

RP054 Toxicological effects of triclosan (TCS), triclocarban (TCC), and their degradation products to a model organism the nematode Caenorhabditis elegans

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Triclosan (TCS) and triclocarban (TCC) are antimicrobials that are widely used in soaps, plastics, clothing, and other household products, and have been detected in air, food, water, terrestrial and aquatic organisms, and humans. TCS and TCC enter the environment everyday through the use of personal care, consumer, and medical products. Once in the environment, TCS and TCC are exposed to ultraviolet (UV) radiation that can degrade these compounds and result in the formation of toxic and carcinogenic products, such as dioxins, furans, chlorophenols, and anilines. The potential environmental health implications of these degradation products and mixtures have not been well understood. Recent studies suggest that TCS and TCC may have adverse effect to aquatic life due to their potential endocrine-disrupting properties. Here we assessed the photolysis of TCS and TCC and potential toxicological effects of TCS, TCC, and their degradation products using a model organism the nematode *Caenorhabditis elegans* (*C. elegans*). To assess the photolysis of TCS and TCC we exposed these compounds suspended in worm media to UVA rays for 1, 3, 5, or 7 days. The toxicity of 7-day UV exposed TCS and TCC increased from 3.65 mg/L to 0.95 mg/L and 0.91 mg/L to 0.04 mg/L, respectively. The UV exposed TCS and TCC were further examined for toxicological effects using organismal and molecular endpoints, including lethality, reproduction, lifespan, hatching rate, germline toxicity, and oxidative stress. Larval or young adult worms were exposed to environmentally relevant concentrations of TCS, TCC, and the UV exposed compounds and examined using above-mentioned endpoints. The UV exposed compounds showed reproductive toxicity at lower concentrations compared to the parent compounds. These findings suggest that TCS and TCC may pose significant health risks to aquatic organisms that increases over time. Our next steps are to characterize the degradation products that were formed during the UV exposure and understand the underlying mechanism(s) of observed reproductive toxic effects of TCS, TCC, and their degradation products.

RP055 The Anti-depressants MK-801 and Desipramine Alter Hemoglobin Expression and Male Production in the Branchiopod Crustacean Daphnia pulex

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Branchiopod crustaceans (e.g. *Daphnia sp*) are subject to environmental sex determination whereby the introduction of males into a population marks the switch from asexual parthenogenetic reproduction to sexual reproduction. The crustacean hormone methyl farnesoate is responsible for programming developing oocytes into male offspring, and its production is initiated by sensory integration of environmental cues in the nervous system and subsequent activation of neuroendocrine pathways. Chemicals in the environment that specifically target the nervous system, such as anti-depressants, may perturb the signaling pathways involved in environmental sex determination, resulting in altered male production. We assessed whether two tricyclic chemicals with anti-depressant activity in vertebrates, MK-801 and desipramine, altered environmental sex determination in *D. pulex* under environmental conditions where male production occurs (10:14 L:D, 18°C). We found that both compounds significantly increased male production in a concentration-dependent manner. Additionally, we observed a concentration-dependent increase in red coloration indicative of hemoglobin expression for both chemicals. As such, we assessed the relative expression of hemoglobin mRNA levels in *D. pulex* across treatments and found that mRNA levels were elevated in treated animals. Our findings suggest that perturbation of male production via neuro-active chemical exposure increases both male production and hemoglobin expression, two physiological responses that can be observed in daphnids in response to sub-optimal environmental conditions. Our results suggest that neuro-active chemicals have the potential to dysregulate environmental sex determination in daphnids. Alterations in sexual reproduction in daphnids may negatively impact population dynamics and disrupt lentic freshwater ecosystems.

RP056 Comparing metabolomic responses in Oryzias latipes to environmentally relevant concentrations of metformin and its metabolite, guanylurea

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Human pharmaceutical wastes threaten many natural processes of non-target aquatic organisms, through the introduction of such contaminants into the environment. One of the most prevalent contaminants is the type 2 diabetic drug metformin. Metformin has been measured in surface waters and wastewater effluents in the ng-µg/L concentration range. Our recent research shows that Japanese medaka (*Oryzias latipes*) exposed to environmentally relevant concentrations of metformin from embryo through 28 days post hatch have a significant decrease in length (mm) and weight (mg) of both males and females when compared to control fish. We demonstrated using radio labelled metformin that about 1% of the waterborne concentration of metformin is found inside both embryo and larval medaka after exposure windows ranging from 24 hours to 7 days. As >90% of metformin is metabolized into guanylurea during the waste water treatment process, its metabolite guanylurea is also a major concern. Guanylurea is found in the environment in higher concentrations than metformin, usually in the µg/L concentration range in surface waters. Metabolomics is emerging as an efficient method for understanding sub-lethal effects on organisms by assisting in determining the biochemical mode of action in response to exposure to a particular contaminant. This study compares the effects of metformin and its metabolite guanylurea to fish early-life stages, in Japanese medaka, including length, weight, and changes to the fish's metabolome.

RP057 Swim bladder inflation failure of larval Japanese medaka (Oryzias latipes) as a toxicological endpoint

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Failure of swim bladder inflation can have serious long term effects on fish populations. Many environmental contaminants can inhibit swim bladder inflation of larval fish. Previous studies in our laboratory have determined that embryonic exposure to pharmaceuticals including 17α-ethinylestradiol, levonorgestrel, and diclofenac, both alone and in mixtures, can impair swim bladder inflation of Japanese medaka (*Oryzias latipes*). We have correlated swim bladder inflation failure of larval medaka with increased mortality following hatch. How embryonic exposure to xenobiotic compounds are able to cause swim bladder inflation is not fully understood; however it is possible that compounds are able to cause their effects through a disruption of embryonic cell signalling or through an inhibition of surfactant production. The effects of embryonic exposure to 17α-ethinylestradiol, levonorgestrel, and diclofenac on gene expression related to swim bladder inflation will be discussed. We established that these pharmaceutical compounds significantly inhibited the expression of genes related to the formation of the three layers of the swim bladder (epithelial, mesenchyme, and outer mesothelium), with levonorgestrel causing the most significant effects on expression. Two of the pharmaceutical compounds, 17α-ethinylestradiol and diclofenac, were also found to disrupt pbx1b, a gene involved in surfactant production.

Emerging Environmental Chemistry – Trends, Transformations and Fate of Organic Environmental Contaminants

RP058 Exposure of Parabens, Benzophenones and Triclosan in Children with Autism Spectrum Disorder

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Autism spectrum disorder (ASD) is a complex neurodevelopmental condition with lifelong impacts. It is contributed by genetic and environmental factors. The prevalence of ASD rised significantly during the last decades. Resent years, the relationship between ASD and environmental factors are highly regarded. It has been proved that phthalates and Bisphenol A are closely related to ASD. However, the effects of other emerging endocrine disrupting chemicals which are frequently detected in human body fluid on ASD are still limited. In this study, 34 children with autism spectrum disorder and 28 health children were recruited and plasma samples were collected. Serum concentrations of total 22 compounds belong to bisphenols, parabens, benzophenone(BP)-type UV filters and triclosan were detected by using high-performance liquid chromatography equipped with electrospray triple quadrupole mass spectrometer (HPLC-MS/MS). Serum concentrations of 2-hydroxy-4-methoxy benzophenone (BP-3), triclosan (TCS), Bisphenol S (BPS), Bisphenol F (BPF), 2-hydroxynaphthalene and 2-hydroxyphenanthrene in children with autism spectrum disorder were significantly lower than those in healthy controls. Concentration correlations between different conpounds in autistic and non-autistic children were also analyzed and differences were observed. The results suggested that these chemicals may have a role in the pathogenesis of ASD or the metabolism of these chemicals are influenced by ASD.

RP059 Human Exposure to Disperse Dyes in House Dust: Examining TRβ Inhibition

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Disperse dyes are hydrophobic organic colorants used in synthetic fabrics such as polyester. We have recently discovered occurrence in household dust samples of several halogenated, azobenzene-based disperse dyes: Disperse Blue 60, Disperse Blue 373, Disperse Orange 25, Disperse Orange 37, Disperse Orange 61, and Disperse Violet 93; we have also discovered the suspected dye metabolites 2-bromo-4,6,-dinitroaniline and 2,6-dibromo-4-nitroaniline in these samples. These compounds were present at ng/g concentrations, similar to and correlated with measured levels of brominated flame retardants (BFR). Azobenzene-based disperse dyes have been implicated both as mutagens and as contact allergens, warranting detailed study into the implications of human exposure in the indoor environment. BFRs have previously been implicated as thyroid disrupting chemicals due to their abilities to mimic thyroid hormones and disrupt signaling pathways. Based on the structure and halogenation of the identified dyes, their structural similarities to BFRs, and their co-application with BFRs, we hypothesized that these dyes would also interfere with thyroid hormone receptors (TRs) signaling. We used a stably transfected cell line (GeneBLAzer®UASbla HEK 293T cell line, Thermo Fisher) to examine agonistic and antagonistic behavior of the identified dyes, and their potential metabolites, on TR beta (TRβ). Cells were exposed to a given dye at concentrations ranging from $1.55x10^{-3}$ M to $1.18x10^{-4}$ M. Our preliminary results show statistically significant antagonisms (95% confidence) of TRβ by Disperse Orange 25, Disperse Orange 37, and 2,6-dibromo-4-nitroaniline at concentrations > $1.28x10^{-3}$ M, and by Disperse Blue 373 at concentrations > $4.70x10^{-4}$ M. Cytotoxicity was also induced by Disperse Blue 373, Disperse Orange 25, and 2,6-dibromo-4-nitroaniline at concentrations > $1.28x10^{-3}$ M. Further trials are currently in progress to substantiate and expand these results. This study reports the first occurrence of disperse dyes in house dust and suggests they may be inhibiting thyroid signaling. Further research of these compounds is warranted.

RP060 Occurrence of phthalates and bispehenols in personal care and consumer products from Korea: Implication of exposure to women and their infants

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The esters of phthalic acid (phthalates) and bisphenol analouges (BPs) have been widely used for several decades in personal care and consumer products. Despite high consumption and widespread use of these contaminants in our daily life, little is known about contents of phthalates and BPs in many commercial products. In our study, sixteen phthalates and eight BPs were determined in 166 personal care and consumer products, which are comprised of 148 products for women and 18 baby care products,using GC/MS/MS and LC-MS/MS. Among 16 phthalates, DEHP showed the highest detection rate (63%), and followed by DnBP (43%), and DiBP (39%). DCHP, DiHpP and DnOP were rarely detected in all of the product samples (< 3%). BPs were less frequently detected than phthalates; BPA (34%) was most frequently detected, followed by BPAF and BPB (24%). Total concentrations of phthalates and BPs ranged from < LOQ to 46,400 (mean: 310) µg/g and from < LOQ to 2450 (mean: 23.2) ng/g, respectively. DnBP concentration was the highest (mean: 279 µg/g), and DEP (mean: 22.0 µg/g) and DEHP (mean: 5.6 µg/g) showed relatively higher than other chemicals. Most of BP analogue levels were generally close to sub-ng/g level. In order to gain usage frequency information (times/day) and amount of usage (g/time) of the Korean women and infant, a questionnaire survey was conducted in Korea. In this study, the estimated dermal intakes of phthalates via the use of personal care and consumer products were 24.6 µg/kg body weight/day and 0.39 µg/kg body weight/day for Korean women and infants, respectively. The dermal intakes of BPs were estimated to be 0.30 ng/kg body weight/day and 0.004 ng/kg body weight/day for Korean women and infants, respectively. Considering no comprehensive data on phthalates and bisphenol analouges in commercial products for human exposure in Korea, further studies are required to assess the exposure status of these contaminants with multiple exposure sources, such as diet, dust, inhalation, drinking water, and contact with other products (e.g. thermal receipts).

RP061 Monitoring for pesticide drift of herbicides 2,4-D, acetochlor, atrazine, and glyphosate at Iowa farm households

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A large proportion of Iowa (92%) is agricultural farmland, and 77% of this land was treated with herbicides in 2012 (USDA). Workers, civilians, and wildlife near these fields are at risk of pesticide exposure from drifting chemicals during treatment season. We have examined more than 300 case files of pesticide drift (Iowa Department of Agriculture and Land Stewardship) from 2010-2016 and found that 87% of pesticide drift cases were related to agriculture, 64% of cases took place in the spring, and 67% involved ground application with an implement sprayer or tractor. In addition, 89% of pesticide drift cases affected individuals, livestock, and plant life within 150m of the nearest applied field. The most common pesticides reported in case files were 2,4-D, acetochlor, atrazine, and glyphosate. Therefore, from April to June 2017, we deployed 34 polyurethane foam passive air samples (PUF-PAS) outside 10 agricultural households and in 3 urban locations (Iowa City, IA) to monitor for these four most common herbicides using a novel passive air method for pesticides combined with LC/MS/MS analysis. All agricultural households were located < 200m of the nearest applied agricultural field, and urban locations were located >2,500m from the nearest applied agricultural field. Eight of ten surveyed farmers reported use of glyphosate, 5/10 reported atrazine, 4/10 reported 2,4-D, and 4/10 reported acetocholor. Results from chemical analysis is ongoing. A new model using local weather data will be used to determine air sampling volumes. We will examine average airborne levels of these compounds during the 2 month application period in both agricultural and urban locations.

RP062 Distribution and fate of the antidiabetic drug, metformin, in the Columbia River

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Metformin (1,1-dimethylbiguanide)—the most commonly prescribed anti-diabetic drug, worldwide—has been widely observed in surface waters at relatively high concentrations (µg L^{-1}) compared to trace contamination by other pharmaceuticals and personal care products. Metformin is unusual among drugs in that its chemical form remains unaltered by the human body and is excreted largely intact, contributing to its relatively high concentrations in waterways. This is problematic because recent studies have shown that metformin has endocrine disrupting effects in fish, making it a contaminant of emerging concern. To better understand the potential threat to ecosystems and fish populations from metformin, we determined monthly distributions of metformin and its primary breakdown product, guanylurea, along a 170 mile stretch of the Columbia River by high performance liquid chromatography coupled to tandem mass spectrometry. Concentrations of metformin ranged from 13.7—195.5 ng L^{-1}, while guanylurea ranged from 32.6—198.1 ng L^{-1}, with the highest values occurring downstream of the confluence of the Willamette and Columbia Rivers. Detectable concentrations of both contaminants were found at each site examined. With cases of Type II diabetes expected to rise to >350 million people by 2030, preventative strategies to reduce inputs of metformin to surface waters need to be developed and implemented in order to protect critical aquatic habitats.

RP063 Measurement of Levels of Organophosphate Flame Retardants (OPFRs) in the Tampa Bay Area

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Flame retardants are chemicals that are added to many consumer products such as plastics, electronics, textiles, foams, and automobile interiors to pass standardized fire tests and achieve the necessary safety levels. Historically, the most widely used flame retardants were polybrominated diphenyl ethers (PBDEs), although others have been used, including hexabromocyclododecane (HBCD), tetrabromobisphenol-A (TBBP-A), organophosphate flame retardants (OPFRs), decadibromodiphenyl ethane (DBDPE), novel brominated flame retardants (NBFRs) and others. FRs are slowly released into the environment by volatilization and abrasion because some are not chemically bonded with the original material. With the phasing-out of PBDEs, alternative FRs have been increasingly used in recent years. Among the major alternatives are OPFRs. Dust is a good receptor for flame retardants and provides a medium for their transport due to their large specific surface area. Several studies have suggested indoor dust as a major source for human exposure to PBDEs, especially for children who spend more time closer to the floor and who ingest dust via significant hand-to-mouth activity in which they engage. It has also been suggested that this is likely the same for alternative flame retardants, including OPFRs. Therefore, there is a critical need to understand the cycling and fate of OPFRs in indoor environment and to determine exposure in humans. The current study measured levels of OPFRs in indoor dust in the Tampa Bay (Florida) region and calculated exposure rates based on those levels. Levels in different microenvironments were compared, including urban versus suburban, and residential homes versus public buildings.

RP064 Current Use Pesticides in the Russian River watershed, California, USA

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The agricultural regions of the Russian River watershed in northern California are subjected to the application of a broad range of current use pesticides, many of which have not yet been monitored in the region. The California Department of Pesticide Regulation's (DPR) Pesticides Use Reporting (PUR) database and Surface Water Protection Program (SWPP) pesticide monitoring prioritization model were used to develop a prioritized list of agricultural pesticides as targets for monitoring. This model utilizes pesticide toxicity benchmarks in water and county use data to run a watershed-based pesticide prioritization algorithm. Other factors informing the final prioritization of target analytes included pesticide properties such as half-life and bioavailability, seasonal and spatial use trends, and emerging information on toxicity and transformation products. In fall 2016, water (dissolved and suspended sediment) and sediment samples were collected at five sites along the Russian Riverand its tributaries. Sites were selected based on spatially explicit data on registered pesticide applications in upstream watersheds in prior summers (2012-2014). The majority of sites were selected to capture agricultural runoff, including downstream of major wine-growing regions. One site downstream of the municipal wastewater treatment plant serving the largest city in the region was also included. Samples were screened for a broad list of target analytes informed by the pesticide prioritization.

RP065 Fecal Matter as a Diagnostic Tool for Measuring Exposure of Freshwater Aquatic Mammals to Anthropogenic Organic Contaminants in Aquatic Environments

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The purpose of this work is to develop and validate a noninvasive method for analyzing exposure of aquatic mammals to anthropogenic organic contaminants (AOCs). This study will evaluate exposure of aquatic mammals to a variety of AOCs that represent a range compound classes and properties that include pharmaceuticals (ibuprofen, acetaminophen, diclofenac & carbamazepine), personal care products (triclosan), pesticides (atrazine), compounds for industrial use (bisphenol-A) and, combustion byproducts (phenanthrene). Despite their increasing use to enhance quality of life, many anthropogenic compounds can have negative effects when they enter the municipal waste systems, survive wastewater treatment, and enter natural environments becoming contaminants. Some AOCs have been reported to interfere with the normal functions of some organisms such as; inhibits reproductive success, disrupts endocrine system, and decrease immune functions. Otters and minks are higher trophic organisms within the continental aquatic food chain, hence determining the exposure of these aquatic mammals to AOCs will help assess the potential for movement and biomagnification of AOCs in the aquatic habitat. However, using traditional methods of tissue, fluid, or organ sampling are highly invasive requiring trapping, anesthetization, or even euthanizing the organism. We aim to develop an analytical method that could be applied to analyze AOCs in the fecal matter to avoid the need for other invasive sampling processes. The analytical method being developed utilizes pressurized liquid extraction (PLE) to extract target analytes of interest from the fecal matter. A cleanup and preconcentration process using solid phase extraction and evaporation prior to quantitative analysis by GC/MS was employed. Current method development has experience significant matrix effects that use of an internal standard has not accounted for. As a result standard addition is been utilized for quantification. The standard addition approach appears promising with initial recoveries ranging from about 50 to 120% for most target analytes. When fully validated the method will be applied to fecal samples of aquatic mammals recovered from natural habitats using trained canines to identify potential exposure to AOCs. If successful, a new noninvasive analytical method to measure AOCs in the fecal matter of aquatic mammals could be the basis for further research on similar analytical methods for other organisms.

RP066 Influence of life-history parameters on temporal and spatial patterns of persistent organic pollutants in eastern North Pacific gray whales

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The presence of persistent organic pollutants (POPs) in the marine environment is of global concern for marine mammal health. These lipophilic contaminants are known to disrupt reproductive and immune systems and cause developmental dysfunction in terrestrial mammals; similar effects are suspected in marine mammals. The objective of this research project is to examine 1) temporal and geographical variation of contaminant burdens, specifically organochlorines and polybrominated diphenyl ethers (PBDEs), in the eastern North Pacific (ENP) gray whale *(Eschrichtius robustus)* population and 2) longitudinal and average patterns of contaminants in individuals of different life history parameters including sex, age and reproductive status. Although previous research conducted in the 1980s-1990s reported POPs in gray whale blubber, subsequent analyses are lacking to establish long-term, longitudinal and geographical trends of contaminants in this protected species. There is also a need to investigate the influence of life-history parameters on these trends in order to enhance interpretation of contaminant loads in gray whales. This project provides the first report of PBDE levels in gray whales. Approximately 150 gray whale blubber biopsy samples collected from 2003-2016 along the ENP gray whale migration route from Alaska to Baja California have been obtained through multi-institutional and international collaborations. Represented in these samples are individuals of different sex and reproductive states. Nine females were resampled yielding multiple samples from the same individuals during different stages of their reproductive cycles. One male was resampled across different years allowing for longitudinal analysis of his contaminant load. Following the methodology described by Sloan et al. 2014, contaminants and lipids were extracted from tissue samples using dichloromethane with Accelerated Solvent Extraction. Sample cleanup was conducted using silica/alumina column chromatography and size-exclusion High-performance Liquid Chromatography. Contaminants were identified and quantified (lipid normalized) using Gas Chromatography/Mass Spectrometry. Contaminant analysis of our biopsy sample collection is currently underway.

RP067 Bioaccumulation of Carbamazepine in Hornworms by Herbivory of Tomato Plants Grown in Soil Fortified with Carbamazepine

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The use of biosolids (treated sewage sludge obtained from waste water treatment facilities) as a means of replenishing nutrients to crop fields is well established. A significant body of research has focused on pharmaceuticals being transferred to crop plants; however there is not as much describing whether the pharmaceuticals are further transferred through terrestrial ecosystems to higher trophic levels. Carbamazepine (CBZ) is a commonly found pharmaceutical in biosolids and has been shown to be taken up by tomatoes. This study aims to determine the transfer of CBZ from tomato plant leaves to hornworms to illustrate the potential for pharmaceuticals to be transferred through increasing trophic levels.

Tomatoes grown under greenhouse conditions in CBZ fortified soil were used as food for hornworms that were collected for analysis at the fifth instar and also collected after pupation as adult hawkmoths. CBZ was detected in the tomato leaves (22ng/g ww), hornworm tissue (3.17ng/g ww) and hawkmoth tissue (1.33ng/g ww). CBZ present in the hornworm and hawkmoth tissues supports the transfer of pharmaceuticals through trophic levels. Further, hornworms that attain CBZ through their diet conserve CBZ through metamorphosis. This may lead to negative behavioral effects. The decreasing concentration trend suggests that bioaccumulation and biomagnification are not occurring within these organisms.

RP068 Species transformation and adsorption of dimethylmonothioarsinic acid (DMMTAV) and dimethyldithioarsinic acid (DMDTAV) in soil

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Toxic arsenic species, DMA^V, can be transformed into highly toxic dimethylmonothioarsinic acid ($DMMTA^V$) and further into dimethyldithioarsinic acid ($DMDTA^V$) under sulfidic condition, therefore DMA^V was recently classified as carcinogen. In our previous work, DMA^V thiolation process (DMA^V > $DMMTA^V$ > $DMDTA^V$) were significantly affected by chemical environmental condition and its accumulation in environmental media were proposed through reverse process ($DMDTA^V$ > $DMMTA^V$). This study aims to determine adsorption possibility of the dimethyl and thiol arsenic species onto soil, in which case DMA^V and $DMMTA^V$ inflow. Due to standards of $DMMTA^V$ and $DMDTA^V$ are commercially unavailable, those were synthesized using our modified referenced methods and will be analyzed using our developed analytical method. Briefly, purity of synthesized $DMMTA^V$ and $DMDTA^V$ were 91% and 88%, respectively, can be maintained 13 weeks with < 4% and < 10% species alteration. Moreover, developed analytical method under a single column based on isocratic elution in HPLC-ICP-MS system can determine iAs^V, iAs^{III}, MMA^V, DMA^V, $DMDTA^V$, and $DMMTA^V$, simultaneously. Soil batch sorption experiments will be conducted by adding known initial concentration of each DMA^V, $DMMTA^V$ and $DMDTA^V$ stock solution. Every 24 h intervals, supernatant will be collected and analyzed arsenical species. Result will infer input species transformation and stability changes, includes DMA^V thiolation. In addition, comparing between total concentration of arsenic of soil before and after batch test can be assumed whether adsorption of DMA^V and $DMMTA^V$ onto soil.

RP069 Rhizosphere effects on lamotrigine accumulation by wheat plants

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Irrigation with treated wastewater and soil amendment with biosolids are increasingly common practices in agricultural systems, and result in exposure of crop plants to a variety of ionizable organic contaminants (IOCs). Crop plants may take up and accumulate IOCs, but our understanding of the variables that control their phytoavailability contains many gaps. Soil pore water pH determines IOC speciation and hence availability for root uptake. Plants can change the pH of the soil immediately surrounding their roots up to 2 units in either direction by secreting H^+, OH^-, HCO_3^-, and organic acids in response to nutrient availability. We have shown that wheat bioaccumulation of the cationic anti-epileptic drug lamotrigine ($pK_a = 5.7$) correlates with the neutral fraction of lamotrigine in a model rhizosphere composed of silica sand. Though the initial pH was 5.7 for all plants, by the end of the exposure period, plants grown on only nitrate had a significantly higher rhizosphere pH and significantly more lamotrigine in their aerial tissues than plants that were also grown on ammonium. This trend did not hold for the neutral anti-epileptic drug carbamazepine. Having demonstrated the principle that rhizosphere pH impacts the root uptake of a model IOC, we have initiated studies in several natural soils to examine the effect of rhizosphere responses to nutrient availability on the uptake of lamotrigine and carbamazepine. In these systems, rhizosphere pH impacts both sorption and uptake of IOCs. Implications of our results on the bioavailability and accumulation of other IOCs will be discussed.

RP070 Photolysis of 2,4-dinitroanisole and its metabolites in Arabidopsis leaves

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New insensitive munitions explosives, including 2,4-dinitroanisole (DNAN), are replacing traditional explosive compounds to protect soldiers and simplify transport logistics. Despite the safety benefits of these new explosives, feasible strategies for cleaning up DNAN from soil and water remain elusive, and the risk associated with ecosystem and human exposure remain unknown. Here, we evaluate the metabolism of DNAN by the model plant *Arabidopsis* to determine whether DNAN is taken up and transformed by plants. Furthermore, to determine the impact of photolysis on the phytoremediation of contaminants, we photolyzed DNAN and its plant metabolites within *Arabidopsis* leaves. We used high resolution and tandem mass spectrometry in combination with stable-isotope labeled DNAN to confirm 11 sugar- and glutathione-conjugated DNAN metabolites in *Arabidopsis*. The identified metabolites were subsequently photolyzed within leaf tissue under simulated sunlight, and ^{15}N-DNAN yielded $^{15}NO_2$ in leaves. Photo-denitration presumably decreases the toxicity of DNAN and its metabolites and suggests that photolysis inside leaves may represent an unexplored mechanism important to phytoremediation and to the transformation of emerging contaminants in the environment.

RP071 Effects of Rhizosphere Bacteria (A. brasilense, P. flurorescens and P. pseudoalcaligenes) on Carbamazepine Uptake from Reclaimed Water in Corn

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Modern municipal wastewater treatment end products, wastewater effluent and sewage sludge, harbor anthropogenic organic contaminates, including the human pharmaceutical carbamazepine (CBZ), a widely prescribed anticonvulsant. Both sludge and wastewater are applied increasingly for irrigation and as soil amendments to enhance vegetation growth. The literature has established plant uptake of CBZ from hydroponic, soil irrigation, and biosolids-amended systems and have established that plants can uptake CBZ under environmentally relevant concentrations making them potentially available for higher trophic level non-target organisms. The focus of this study was to determine what role mutualistic rhizosphere bacteria *Azospirillum brasilense (AZ)*, *Pseudomonas flurorescens* (*PF*) and *Pseudomonas pseudoalcaligenes (PP)* might play in the uptake of CBZ from a simulated reclaimed wastewater hydroponic system. Corn plants were exposed to a series of three bacteria either alone or in series and nutrient solution supplemented CBZ (2.38µg/L) or a control non-supplemented solution. CBZ concentrations were quantified in corn plants using isotope dilution liquid chromatography-mass spectrometry. CBZ was detected in aerial plant tissue in higher concentrations when the plants were exposed to *PP* and *AZ*. Interestingly, this trend was reversed when *PF* was present, appearing to mitigate CBZ uptake.

RP072 Analysis of Oxilofrine in Citrus Fruits Using Ultrahigh Performance Liquid Chromatography (UHPLC-MS/MS) and Modified QuEChERS Clean-Up

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The objective of this study was to develop and validate an extraction and quantification method to extract the stimulant oxilofrine from a citrus matrix. Oxilofrine is not known to occur naturally in citrus fruits but its structural similarity to synephrine, which also elicits similar stimulatory effects and has been extracted from various types of oranges, strongly suggests that it may be present. Due to the potential adverse health effects of oxilofrine, there is a need for more sophisticated methods to analyse this illegal pharmaceutical in food products. The QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) method has proven effective in analyte extraction from fruit, so it was used to extract oxilofrine along with dispersive solid phase extraction (dSPE) to remove matrix components. Ultrahigh performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) was used for analysis and quantification. Recoveries of oxilofrine ranged from 83.3 and 90.6% using magnesium sulfate, primary secondary amine exchange material and endcapped c18 sorbents. In a survey of twelve oranges of different types and sources, oxilofrine was found at concentrations from 5.5 to 85.7 ng g^{-1}. The highest concentrations were observed in samples labeled as mandarins, which correlates with synephrine, which occurs at high levels in mandarins and tangerines.

RP074 Online SPE-LC-HRMS for the determination of common UV-blocking chemicals from sunscreen in surface waters

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Human pressure introduces a multitude of anthropogenic signatures in particular those related to direct introduction of contaminants or emission of waste products, which have negative impact in the water quality. Most of these pressures come as a diffuse emission from multiple non-point sources and are difficult to track. Others, however, are more localized and could be directly assessed due to the specific nature of the stressor and the known identity of the target organism. One of those processes relates to the introduction of emergent contaminants such as components of sunscreen lotions and sprays used during aquatic recreational activities, also known as “human tourism”. The increasing use of sunscreen to prevent UV radiation damage to the skin has led to their widespread occurrence in surface water influenced by human activity, which adversely affects important marine and nearshore organisms such as corals, phytoplankton and sponges. Ecotoxicology studies have shown that UV filters act as environmental estrogens and antiestrogens, cause reproductive disruption and affect the thyroid axis. In this study, an online solid phase extraction (online SPE) in combination with high-performance liquid chromatography coupled to high-resolution mass spectrometry (HPLC-HRMS) method was developed and validated for the simultaneous determination of 11 UV filters in water. Target compounds included the most common ingredients of sunscreen formulations sold in the US p-Benzoylphenol, 4,4-Dihydroxybenzophenone, benzocaine, benzophenone-D10, oxybenzone-D5, oxybenzone, dioxybenzone, octocrylene, enzacamene, benzoresorcinol, 2,2,4,4-Tetrahydroxybenzophenone and avobenzone. The analysis was performed in full scan of 140,000 resolution in positive ion mode using a Q-Exactive. The chromatographic separation was performed in a Hypersil gold aQ column using methanol and 0.1% formic acid as mobile phase. The time required for the analysis was 9 min and the limits of detection were as low as 5 ng/L for most compounds. The procedure proposed provides a fast, accurate and robust analytical method, with low consumption of organic solvent and the detection of UV-filters at low part per trillion levels (ng/L). Ultimately, the method will be used to measure UV filters in surface and reclaimed water from the Biscayne Bay area in order to better understand the distribution and fate of UV filters in the aquatic ecosystem.

RP075 Screen to detect the transformation products of pharmaceuticals in water-sediment systems

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The dissipation half-live values (DT_{50}) derived from kinetic analysis of OECD 308 experimental data is used in the Environmental Risk Assessment (ERA) to characterize fate and to support the classification of an active pharmaceutical ingredient (API) for potential persistence in the environment. As per guidance, identification of transformation products (TPs) is triggered if present at >10% of the initially applied dose. Chromatographic data from pre-clinical studies indicate that peaks attributable to metabolites and to parent API can often elute closely, requiring vigilant separation methodology. Misidentification of a TP as parent API due to incomplete chromatographic separation may result in overestimating the amount of parent found and subsequently incorrectly classifying the API as environmentally persistent. Also, any TP misidentified as parent API will themselves not be quantitated nor identified. Further hindrance to TP identification in the OECD 308 test design concerns the recommended sediment-water ratio, which experience has shown favors mass distribution to the sediment phase, resulting in high levels of non-extractable residues (NER). Here we report on an approach developed to screen for water-sediment TPs using a modified version of the OECD 309 Aerobic Mineralization in Surface Water Simulation Test. The objective of this study is to develop a screen that can leverage the most TPs in the shortest timeframe. Adaptations of the OECD 309 protocol investigated were 1) elevating the temperature to increase the rate of transformations and 2) use of pelagic vs. suspended sediment river waters, investigating biodegradation via sediment-associated microorganisms and NER formation. Additional TP profiles were generated using unlabeled APIs with LC/MS/MS analysis, to help establish confidence in a generalized-screen approach, useful for investigating legacy APIs for which radiolabel is not available. TP profiles generated in this study are compared to human and non-clinical metabolism profiles, and transformation mechanisms suggested. Overall, the TP profiles generated are similar to what’s observed in the OECD 308 study. Identifying potential TPs prior to initiating definitive fate studies will allow for optimization of study conditions. Further, development of a TP library using this screen will help to contribute to the understanding of, and improving predictions of environmental transformations.

RP076 Impact of On-site Wastewater Treatment Systems (OWTSs) on Trace Organic Contaminant (TOrC) Mixtures in Minnesota Lakes

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On-site wastewater treatment systems (OWTSs) are common in Minnesota lakeshore developments without access to centralized treatment facilities. Recent studies have hypothesized that these systems and/or other non-point sources, are responsible for trace organic contaminant (TOrC) occurrence in surface waters throughout the state. This research evaluated concentrations of known TOrCs in five Minnesota Lakes at near-shore environments both within (Residential) and outside (Reference) proximity of OWTSs. Sampling locations also served as sunfish spawning habitats concurrently studied for adverse biologic effects. Two-group assessments demonstrated significantly different TOrC compositions in grab samples of Residential and Reference porewater. A post-sampling analysis of proximity corroborated site distinctions. Passive sampler extracts were analyzed using high resolution mass spectrometry (HRMS) to characterize unknown TOrCs present during spawning season. A comparison of toxicology and HRMS data will assess the impact of TOrC mixtures on local sunfish.

RP077 Sorption of Neonicotinoid Insecticides to Granular Activated Carbon during Drinking Water Treatment

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Neonicotinoid pesticides (neonics) are among the most widely used pesticides in the world due to their targeted toxicity towards insects. The widespread use of neonics has led to their detection in waters across the U.S., including those used as drinking water sources. Although neonics exhibit relatively low toxicity towards mammals, formation of toxic metabolites is an exposure concern. Previous studies comparing neonic detection at the University of Iowa and Iowa City drinking water treatment plants have identified granular activated carbon (GAC) as a possible method for removing the neonics imidacloprid, clothianidin, and thiamethoxam from drinking water. The goal of the present study was to determine the efficacy of GAC to remove neonics from drinking water. Adsorption isotherms were conducted with imidacloprid, clothianidin, and thiamethoxam to assess their affinity towards GAC. Desorption tests were also conducted using five-year-old used GAC from the Iowa City drinking water plant to determine the likelihood of neonic desorption and concentrations of neonics on GAC granules. Results indicate neonics (despite high polarity / solubility) are readily adsorbed to GAC and minimally desorbed in buffered solutions at environmental pH. The greatest extent of neonic desorption occurred in solvents of medial polarity (acetonitrile, acetone, and dichloromethane). In solvents with the greatest desorption of neonics from GAC, concentrations of up to 600 ng neonic/g GAC were determined. These sorption and desorption tests suggest the long-term use of GAC is a viable option for neonic removal from drinking water. Nevertheless, over time, GAC filters are prone to biofilm formation, which may enhance the biotransformation of adsorbed neonics into metabolites of enhanced mammalian toxicity. Studies are currently underway concerning the biotransformation of these neonics in environmental and engineered systems that contain electron transporters such as GAC.

RP078 Identification and quantification of caffeine, acetaminophen, and sucralose as wastewater tracers using an online SPE LC-ESI-HRMS

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Sucralose has been widely used to determine wastewater inputs into environmental systems. Due to its high stability and persistence, it's difficult to establish if the high amounts of sucralose found in the environment are or are not from recent wastewater intrusions. The analgesic acetaminophen (paracetamol) is commonly used as an over-the-counter (OTC) medication, being mostly excreted as metabolites by sulfate and glucuronide conjugation. Caffeine is widely distributed around the world being a major human dietary component. Caffeine and acetaminophen are ubiquitous pollutants in the aquatic ecosystem and since these compounds have short half-lives in water, they would represent good tracers of recent anthropogenic inputs into the environment. To this end, in this study, a new analytical method for the determination of sucralose, acetaminophen, and caffeine has been developed and validated on a Thermo Q Exactive Orbitrap mass spectrometer. An online solid phase extraction was performed on a Thermo Scientific HyperSep Retain PEP 20 mm column followed by separation in a reversed phase Thermo Hypersil Gold C18 column with acetonitrile and 0.1% formic acid as the mobile phase. Good linear responses were observed in the range of 5-5000 pg/mL ($R2 >0.99$). The developed method required minimal sample preparation and an injection of 10.0 mL of water sample, which allowed method detection limits (MDLs) as low as 2, 3, and 22 pg/mL for sucralose, acetaminophen, and caffeine respectively. All results displayed a good degree of reproducibility and repeatability, with relative standard deviations (RSD) of less than 15% for all compounds. The method was employed in surface and reclaimed water collected near Biscayne Bay Campus at Florida International University in order to evaluate the occurrence and variability of these compounds in water and establish possible correlation between sucralose, caffeine and acetaminophen.

RP079 Using Nitrate Source Analysis to Understand Groundwater Quality in Chippewa County, Wisconsin

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Chippewa County, in western Wisconsin, has a unique historical set of groundwater quality data. The county conducted extensive groundwater sampling of private wells in 1985 (715 wells) and 2007 (800 wells). In 2016, they collaborated with UW-Extension and UW-Stevens Point to evaluate the current status of groundwater quality in Chippewa County by sampling of as many of the previously studied wells as possible. Nitrate was a primary focus of this groundwater quality inventory. Of the 744 samples collected, 60 were further analyzed for chemical indicators of agricultural practices and septic waste, two major sources of nitrate contamination. Wells for nitrate source analysis were selected from the 2016 participants based upon certain criteria. Only wells with a Wisconsin Unique Well Number were considered. Next, an Inverse Distance Weighting tool in ESRI ArcMap was used to assign density values. Two-thirds of the wells were selected in higher density areas and one-third in lower density areas. Equally prioritized was an even distribution of nitrate – N concentrations, with half of the wells having nitrate – N concentrations higher than 10 mg/L and about half with concentrations between 2 and 10 mg/L. All wells with a WUWN and nitrate – N concentrations greater than 20 mg/L were selected. Nitrate and source indicator concentrations were then evaluated based on percent agricultural land use and septic system density. The results of the nitrate source analyses will aid in determining temporal changes and spatial relationships of groundwater quality to soils, geology and land-use in Chippewa County.

RP080 Recycled crushed-glass for sub-surface passive filtration of wastewater contaminants in northern prairie climates

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Global concerns about wastewater contaminants and their effects on water quality are continuously increasing. Chronic effects of pharmaceuticals in aquatic life are not fully understood and the presence of antibiotic-resistant gene(ARG)-bearing organisms in the environment could promote outbreaks by antibiotic-resistant pathogens. To better understand the efficacy of wastewater treatment in northern prairie climates, we characterize the performance of recycled crushed glass and sand in a four-cell passive sub-surface filter for a municipal wastewater treatment facility in Dunnottar, Manitoba with special interest in biofilm characterization (i.e. nitrifying and denitrifying bacteria) and the attenuation of polar pharmaceuticals, antibiotic resistant genes and toxicity using two hydraulic retention times, during the Summer of 2017. Wastewater treatment in this facility is managed by the local municipality and is performed by a two-lagoon system with subsequent passive subsurface filtration. Preliminary results of this ongoing work are discussed in this presentation.

RP081 Treat and Toss: Current Consumer Behavior Regarding Medication Disposal

J.S. Punzi, CHPA / Regulatory and Scientific Affairs; A. Brikman, CHPA / Communications; M. Tringale, CHPA

Over-the-counter (OTC) medicines comprise the majority volume of drugs purchased by consumers, but little is known about how consumers dispose of expired/unwanted OTC medicines. Keeping these medications in the home presents increased risk of hazards for children, drug diversion, or improper use. Other research has shown that some disposal methods such as flushing potentially add burden to local wastewater treatment systems. Proper OTC medication disposal is important for household safety and other concerns. CHPA and the CHPA Educational Foundation conducted three surveys to understand and measure consumer awareness, attitudes, and behaviors regarding disposal of expired/

unwanted medicines including a national survey among 1000 adults; a regional telephone survey among 1000 adults; and, a local telephone survey among 500 adults. Regarding disposal behavior, respondents reported similar patterns across all three surveys when disposing expired/unwanted medicine. The medication disposal method most typically used is in-home disposal with 78% in the national survey (58% trash, 20% flush), 48% regional (32% trash, 16% flush), and 42% local (32% trash, 10% flush). A minority of respondents reported using out-of-home disposal options including 32% in the regional survey (25% community take-back programs, 7% pharmacy take-back), 25% local (16% community, 9% pharmacy), and 16% national (10% community, 6% pharmacy). Fewer than two-thirds of respondents across all surveys are very concerned about the importance of medication disposal: 61% in the regional survey were "concerned" about disposal, only 42% national say it's a "very important issue", and only 41% local say it's "a very serious problem". Many people across all surveys are not aware of existing disposal options: 62% in the national survey are not aware of existing community disposal options, 56% regional are not aware of existing community disposal options, and 35% national do not know where to find information about medication disposal. Conclusions: Across the U.S. nationally, regionally, and locally, consumers are preferring the convenience of in-home medication disposal, but too many continue to flush. This may be since too few recognize medication disposal as a serious concern, and too few are aware of their disposal options. Efforts to educate and inform consumers about the importance of proper medication disposal and existing options for proper in-home and out-of-home disposal are needed.

RP082 Seasonal Variations in Pharmaceuticals Sales in Canada from 2011-2014

E. Vijay, T. Huang, D. Hughes, A. McLaughlin, B. Tuteja, Health Canada

Pharmaceuticals are used to treat disease and infection. There is a higher prevalence of specific infectious agents and pathogens during certain seasons, and this may correspond to seasonal variation in the consumption of some pharmaceuticals. This project was undertaken in order to identify those pharmaceuticals that demonstrate significant seasonal variations in sales, and to determine whether such variation is significant enough to impact measured monitoring results in waste water influents. Pharmaceuticals representing a number of key categories such as antimicrobials, analgesics, and anti-histamines were selected since drugs in these categories were thought to be the most likely to demonstrate seasonal variation. Quarterly sales volumes (kg/year) of pharmaceuticals in Canada from 2011 to 2014 were extracted from a proprietary database which compiles total sales volumes for individual ingredients for the whole of Canada. Quarterly pharmaceutical sales volumes were identified in accordance with the four seasons in Canada: Q1 winter (Jan.-Mar.), Q2 spring (Apr.-Jun.), Q3 summer (Jul.- Sep.) and Q4 fall (Oct.-Dec.), respectively. Some pharmaceuticals were observed to demonstrate increased seasonal sales that appear to correlate with an increasing prevalence of certain diseases or infections. A number of factors were identified, such as bulk buying and drug shortages that can complicate the correlation of sales volumes with consumption. An analysis of pharmaceutical sales is helpful in understanding pharmaceutical use and to identify those which may demonstrate significant variation in consumption and environmental detections according to season. Possible seasonal variability in pharmaceutical consumption is a factor to consider when interpreting environmental monitoring data and potentially important when modelling environmental concentrations. Ideally monitoring of environmental concentrations should be carried out year round in order to better capture these seasonal fluctuations in influent concentrations due to variation in consumption patterns.

RP083 Determination of the identity and toxicity of ultraviolet filter chemical (UVFC) octyl dimethyl para-aminobenzoic acid (OD-PABA) photoproducts

L. O'Connor, J.N. Maung, M.G. Paulick, L.A. MacManus-Spencer, Union College / Chemistry

Chemicals are a very big part of our lives and have both harmful and beneficial aspects, but would anyone think that everyday products such as sunscreen or cosmetics would have harmful chemicals in them? Organic ultraviolet filter chemicals (UVFCs) are the active ingredients in most sunscreen products and in some cosmetics. These chemicals are used to protect the skin from the damaging effects of sunlight exposure. However, when these products are used, the chemicals end up in the environment. Traces of organic UVFCs have been found in our water, sewage, fish and even humans. When the UVFCs are exposed to sunlight, many degrade and little is known about the environmental fate and potential toxicity of the resulting photoproducts. One commonly used UVFC we are interested in is octyldimethyl para-aminobenzoic acid (OD-PABA), which readily degrades in sunlight. The most common products that contain OD-PABA are lipsticks and lip balms. Previous work has investigated the photochemical degradation of OD-PABA, and the structures of photoproducts have been proposed. This study focuses on investigating the mechanism of OD-PABA photolysis, as well as the isolation and complete characterization of its photoproducts. Direct photolyses of OD-PABA in pure water have been conducted. To simulate "real-world" conditions in a controlled way, indirect photolyses in water with well characterized dissolved organic matter (DOM) were also conducted. DOM absorbs sunlight and transfers energy to OD-PABA or to dissolved oxygen, which may enhance the degradation of OD-PABA.

RP084 Assessing the impact of atmospheric chemistry on the effectiveness of adulticide application in urban areas

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The current spread of mosquito-borne diseases (e.g. Zika Virus) has increased our effort to control mosquito populations throughout urban areas, specifically in more susceptible areas prone to outbreaks (i.e. Southeastern US). Adulticides applied using foggers are the main strategy to control adult mosquito populations in major urban areas such as Houston, TX. Harris County Public Health Mosquito Division (PHMD; the third largest U.S. county), which serves Houston, TX, typically applies malathion and other adulticides around sunset. However, current approaches do not consider the unique chemistry of the urban atmosphere, which potentially has enhanced oxidation processes. An atmospheric field campaign conducted in Harris County by Baylor University measured nighttime atmospheric half-lives of malathion 40-90% lower (< 24 h) than malathion half-lives measured in agricultural settings (1.6 to >9 days); these half-lives were determined using atmospheric concentrations of malathion and its oxidation product, malaoxon, which is 22 times more toxic to humans. Houston, TX has a history of O_3 and NO_x pollution, which enhances nitrate radical production: O_3 and nitrate radical are both known to oxidize malathion. Periods of high NO_x and O_3 concentrations in Houston coincides with peakmosquito season (Jul-Sept), and therefore peak adulticide usage. The goal of this research is to better understand how urban atmospheric composition affects the oxidation rate of malathion, and whether oxidation of malathion to malaoxon occurs in the gas or particle phase.

RP085 Kinetic modeling of sunlight assisted degradation of trace organic substances: p-cresol and fufuryl alcohol indirect photo-oxidation by singlet oxygen

H. Vo, University of Arizona / Chemical and Environmental Engineering

In natural sunlight, effluent organic matter (EfOM) in treated wastewater acts as a sensitizer for the generation of reactive oxygen species (ROS) that contribute to the photo transformation of trace organic contaminants. Two model chemical targets—*p*-cresol and fufuryl alcohol (FFA)—were used to characterize the mechanism and kinetics of ROS-dependent reactions sensitized by EfOM in the UVA, an artificial sunlight source. Results show that UVA-excited EfOM intermediates are not directly responsible for observed *p*-cresol or FFA transformations, and the role of hydroxyl radicals is negligible. Singlet oxygen was responsible for essentially all observed transformations in these experiments. From previous work by others and our own experimental results, a comprehensive mechanism was proposed with a complementary kinetic model to predict the trajectories of *p*-cresol and FFA photo transformations in wastewater effluent. Simulations are built on previously established quantum efficiencies and account for light shading and competitive effects. Agreement between measurements and simulations is excellent.

RP086 Pairing kinetics and compound specific isotope analyses for abiotic reduction of munitions in iron mineral suspensions

J. Strehlau, University of Minnesota / Civil, Environmental, and Geo-Engineering; B. Ulrich, Eawag; T. Hofstetter, Eawag / Environmental Chemistry; W. Arnold, University of Minnesota

Groundwater near munitions manufacturing facilities and military sites is vulnerable to contamination from oxidized explosive compounds, such as the commonly used and toxic explosive RDX. Abiotic reduction by naturally occurring subsurface iron minerals is one promising strategy in the natural attenuation of explosive compounds. This strategy is often investigated using kinetic analyses, but when paired with compound specific isotope analyses (CSIA), the techniques offer insight into both the extent of contaminant degradation and the chemical reaction processes involved. The pairing of these techniques has been used for the assessment of biotic RDX degradation, yet abiotic RDX degradation mechanisms are less explored. In this work, batch reactors were used to evaluate the abiotic degradation of RDX by various nanoscale iron minerals, including goethite, magnetite, and iron sulfides, under different simulated environmental conditions (e.g., varying mineral mass loading, the presence of aqueous Fe(II), and the presence of organic matter). Rates of RDX degradation were quantified using high pressure liquid chromatography, revealing that rates significantly increased with increasing mineral mass loading. On the other hand, rates were independent of the presence of Elliot Soil humic acid (up to 20 ppm organic carbon) and only slightly dependent on the presence of Suwannee River natural organic matter (up to 10 ppm organic carbon). Sequential-spike reactions were also performed to elucidate the effects of continuous RDX degradation in batch reactors, which ultimately varied depending on initial mineral identity. To determine chemical reaction processes involved, stable isotope fractionation will be quantified by CSIA using gas chromatography/isotope ratio mass spectrometry. These results provide insight into the abiotic mechanisms of RDX remediation, improving predictions of natural attenuation performance in contaminated groundwater near affected sites.

RP087 Photodegradation and advanced oxidation of imidazolium, pyridinium, pyrrolidinium, and piperidinium ionic liquid cations

S. Pati, University of Minnesota / Civil, Environmental, and Geo-Engineering; W. Arnold, University of Minnesota

Ionic liquids (ILs) are a class of emerging industrial chemicals, which are expected to be increasingly used by chemical industries as replacements for volatile organic solvents. With increasing usage, there is a potential for ILs to be accidentally released and thus become contaminants in aquatic environments. The most common ILs are composed of organic cations containing quaternary ammonium groups and inorganic or organic anions. The quaternary ammonium cations have been shown to be highly soluble in water and only minimally removed via biological wastewater treatment. While current knowledge of the toxicity of ILs indicates that some quaternary ammonium cations may pose a threat to aquatic ecosystems, little is known about the fate of IL cations in the aquatic environment or in drinking water treatment systems. To investigate the fate of IL cations in surface waters, we studied the direct and indirect photochemical transformation of four common structures of IL cations: imidazolium, pyridinium, pyrrolidinium, and piperidinium. Only reaction with hydroxyl radicals contributed to the transformation of IL cations resulting in estimated half-lives in sunlit surface waters between a few days and several months. Given their persistence in natural aquatic systems, IL cations could potentially be present in source waters for drinking water production. Therefore, additional studies of the reactions of IL cations during disinfection treatments and advanced oxidation processes (AOPs) were performed. While reactions with chlorine and chloramine were negligible, quaternary ammonium cations were readily removed during ozonation as well as with UV/peroxide, UV/persulfate, and UV/chlorine treatments. Under all these conditions, reactions with radical species, such as hydroxyl and sulfate radicals, were largely responsible for the removal of IL cations. Our results indicate that even at low environmental loadings, quaternary ammonium compounds used as IL cations are potential environmental contaminants due to their persistence towards transformation in natural aquatic systems. Furthermore, advanced chemical treatment would be required to eliminate IL cations during drinking water treatment.

RP088 The anti-bactericidal activity of a carbon-nanotube/iron oxide composite material

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Safe use of reclaimed wastewater requires the effective removal of microbial contaminants. Wastewaters are a hotspot for human pathogenic bacteria, all of which must be eliminated to guarantee safe usage. In an answer to this need, we have constructed a carbon-based nanomaterial composed of carbon nanotubes (CNTs) and iron oxides for bacterial deactivation. This material is a potential reusable antimicrobial agent owing to the cytotoxic effects of the CNTs and the magnetic features of the iron oxides. Our main objective was to investigate the anti-bactericidal activity of the composite material. To do so, we chose a model gram negative bacterium – *Escherichia coli*. Less than 4% of viable *E.coli* remained in solution following exposure to the composite at all initial *E.coli* concentrations, as determined from the colony forming count method. Removal of *E.coli* was attributed to both direct killing of cells and to the adsorption of live cells by the composite. Post-sonication of samples was performed to examine the release of adsorbed bacteria. At low initial concentration of *E.coli*, no viable cells were released, indicating that all bacteria removed were indeed nonviable. However, as the initial concentration of *E.coli* increased, the percentage removed from solution decreased, demonstrating that the post-sonication treatment triggered the release of adsorbed viable bacteria. This result suggests that direct contact is essential for toxicity of the composite toward bacteria and that there is limited surface area available for the fatal interaction. Toxicity experiments performed with the pristine CNTs and iron oxide nanoparticles emphasized that only the CNTs contribute to the antimicrobial properties of the composite material, compared to the iron oxides that had no effect on *E.coli* concentration. Live/dead fluorescence staining following exposure to the composite revealed that the vast majority of cells were accessible to the impermeable propidium iodide dye, indicating bacterial cell wall damage. Cell deformation and degraded integrity of cells after exposure was also demonstrated using SEM imaging. Regeneration of the composite was achieved using a washing protocol with calcium chloride and distilled water; the composite's performance was unharmed following three sequential exposure cycles. These findings demonstrate the potential use of the composite as a reusable antimicrobial agent.

Oil and Gas Waters – Integrating Analytical Chemistry and Toxicology to Inform Management Decisions

RP089 Evaluation of the toxicity of elevated major ions in surface water contaminated by produced water from oil production

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The disposal or inadvertent release of produced water from oil and gas extraction has the potential to pose risks to the environment. Waste materials may contain elevated concentrations of major ions, other toxic elements from the mined formation, or inorganic or organic additives used in the oil and gas development process. A brine waste pipeline from oil production in the Williston basin was discovered to be leaking into Blacktail Creek near Williston, North Dakota on January 6, 2015. The objective of the current study was to determine potential effects of elevated major ions in the contaminated waters on the survival and growth of a fish (fathead minnow *Pimephales promelas*) and a unionid mussel (fatmucket *Lampsilis siliquoidea*). Short-term (7-day) toxicity tests were conducted concurrently with the two species in a reconstituted water mimicked the background water quality at an upstream reference site (as controls) and in three reconstituted waters mimicked the contaminated site waters at 1, 2, and 4 times of the ion concentrations (i.e., Na, K, and Cl) measured in a contaminated site water. Significant reduction in growth relative to the control was observed in the 4-time-major ion treatment with minnows, and in all three treatments with mussels. The results indicate that (1) the elevated major ions alone in surface water contaminated by the Williston brine spill might affect fish and aquatic invertebrates, and (2) the mussels were more sensitive to the contaminated site water than fathead minnows. This study can be used to inform risk management for waste materials associated with oil and gas production.

RP090 Sublethal and reproductive effects of acute and chronic exposure to hydraulic fracturing flowback and produced water on the water flea Daphnia magna

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Hydraulic fracturing is an industrial process allowing for the extraction of gas or oil. To fracture the rocks, a proprietary mix of chemicals is injected under high pressure, which later returns to the surface as flowback and produced water (FPW). FPW is a complex chemical mixture consisting of trace metals, organic compounds, and often, high levels of salts. FPW toxicity to the model freshwater crustacean, *Daphnia magna*, was characterized utilizing acute (48 h median lethal concentrations; LC_{50}) and chronic (21 d) exposures. Neonates exhibited an LC_{50} of 0.19% of full-strength FPW, making them more sensitive than adults, which displayed an LC_{50} value of 0.75%. A decrease in reproduction was observed, with a mean value of 18.5 neonates produced per replicate over a 21-d chronic exposure to 0.04% FPW, significantly decreased from the average of 64 neonates produced in controls. The time to first brood was delayed in the highest FPW (0.04%) treatment. Quantitative PCR highlighted significant changes in expression of genes encoding xenobiotic metabolism (*cyp4*) and moulting (*cut*). This study is the first to characterize chronic FPW toxicity and will be used to help understand the impacts, and develop environmental monitoring and risk assessment, of FPW spills.

Community Engagement in Environmental Science – Building Links With Traditional Knowledge and Indigenous Values

RP091 Decolonizing knowledge gathering systems and empowering Native peoples through story telling

C. Sandoval, Indigenous Texas Nature Tours / WFSC; A.C. Camacho, University of Texas Rio Grande Valley / Psychology and Neurology

Our research and community efforts are focused on taking back the classification systems and methods of gaining knowledge from academia in order to empower native peoples, particularly the non academically educated, to use their traditional knowledge together with the community for its betterment. Our specific project, Indigenous Texas Nature Tours, offers a guided nature walk given freely to native peoples, and offered for others who wish to learn, which discuss the native names of plants, birds, and other animals, as well as tell the oral traditions associated with those organisms. These stories and names are often paralleled in various nations' stories and languages, with the research group constantly updating more data and stories to the repertoire. The community's reaction to these tours has been overwhelmingly positive with a common theme in responses of pride in their indigeneity and in their community, both of which receive almost ubiquitous put downs and casual racism in media, as well as in policy. Children tend to be intrigued by the familiarity of the knowledge they are receiving and internalize it to find different connections than even the guides. Adults often have their own stories that they have not shared for years due to the stigma associated with so called tall tales, folklore, and being too "indio". Often, this traditional indigenous knowledge is perceived as not credible, or biased due to the source. These stories are invaluable additions to our knowledge base and are further shared by us to others in the community. In this way we connect our academic expertise of wildlife and biology to indigenous knowledge.

RP092 Understanding and predicting fish mercury levels in the Dehcho region

H. Swanson, University of Waterloo / Biology; G. Low, Dehcho Aboriginal Aquatic Resources and Oceans Management; M. Evans, Environment and Climate Change Canada / Aquatic Contaminants Research Division; B. Branfireun, University of Western Ontario / Biology Centre For Environment and Sustainability; M. Simba, Kaa gee Tu First Nation

Dehcho First Nations, NT, Canada, are concerned about levels of mercury in food fishes such as Northern Pike, Walleye, and Northern Pike. In some traditional fishing lakes, mercury levels are high enough to have led to consumption advisories. Fishers, community members, regulators, monitors, and scientists want to understand why fish mercury levels are relatively low in some lakes but higher in others, and why fish mercury levels are increasing in some lakes but stable in others. By understanding the dominant drivers of fish mercury in the Dehcho, we can more accurately predict how climate change and resource development may affect fish mercury. From 2013-2015, 8 Dehcho lakes were sampled for fish, benthic invertebrates, zooplankton, and sediment. We determined fish mercury levels, as well as fish age, size, trophic level, and food source. Water chemistry samples were also analyzed for a suite of variables. Results indicate that Lake Whitefish are safe to eat in all lakes, whereas Northern Pike have intermediate mercury levels and Walleye have the highest mercury levels. Mercury differences among species appear to be best explained (as expected) by fish size, age, and trophic level. Mercury differences among lakes are more complicated. In Walleye, size-standardized differences in mercury among lakes are best predicted by concentrations of chlorophyll-a (negative). In Northern Pike, size-standardized differences in mercury among lakes are best explained by indicators of catchment weathering and habitat use. These and other results will be summarized, as well as our successes and lessons learned with risk communication, youth engagement, and joint University-First Nations sampling teams.

RP093 Tox on Tap: How to bridge the communication gap between scientists and the public (and somehow make it fun)

E. Maloney, K. Raes, C. Watts, University of Saskatchewan / Toxicology Centre; S.E. Crawford, RWTH Aachen / Institute for Environmental Research, Environmental Analysis; D. Green, University of Saskatchewan / Toxicology Centre

Scientific communication is difficult, especially in an age where environmental issues are growing increasingly complex. Though information is more readily available than ever (via media and internet sources), there has been an increasing amount of misinterpretation and misunderstanding of scientific research by the public. This is primarily due to a breakdown in communication between scientific researchers and non-scientists. We attempt to bridge this communication gap through Tox on Tap (ToT). ToT is a non-profit, volunteer-run initiative to bring together scientists and the local community to discuss topical issues in toxicology and environmental science. Hosted in a local pub, scientists from academia, government, and private industry are invited to discuss their areas of expertise, including low-dose radiation exposure, storm-water runoff, and pesticides. In these events, we attempt to foster an informal and friendly atmosphere to encourage audience participation and education about current scientific issues. We would like to share our successful stories of effectively communicating science, inspiring the public through our events, increasing perception of the scientific community, and somehow making it all fun.

Fate and Effects of Metals – Regulatory and Risk Assessment Perspective

RP095 Accumulation, distribution and toxicity of heavy metals in sediments received effluents from agrochemical and traditional industries, Lake Edku, Egypt

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Heavy metal accumulation in sediment threatens adjacent ecosystems due to the potential of metal mobilization and the subsequent uptake into food webs. Here, contents of heavy metals (Mn, Cd, Fe, Zn, Cr, Cu, Ni, Pb, and Hg) were determined for trace metals from aquatic environment were collected from twelve locations in Lake Edku, during 2014 and 2015. that received sewage discharged from agrochemical and traditional industries. The highest concentrations of metals, except Fe and Mn were detected at Al-Khairy drain which in fact joined to three sources of drainage waters coming from El-Bousely, Edku and Damanhour Sub-Drains, Beheira governorate which transport domestic, agricultural and industrial wastewater. We used principal component analysis (PCA) to determine the metal distribution in relation to environmental factors such as pH, EC, and organic matter (OM) contents in the lake Edku. While water PCA were categorized discharged metals into three groups that implied potential origins of contamination, sediment PCA only indicated a correlation between metal accumulation and OM contents. Such discrepancy in metal distribution between lake water and surface sediment highlighted the significance of physical-chemical properties of sediment, especially OM, in metal retention. Moreover, we used The Pollution Load Index (PLI) and the enrichment factor of the study area (EF mean values) have the order of $EF_{Mn} > EF_{Pb} > EF_{Cu} > EF_{Cr} > EF_{Zn} > EF_{Ni}$. According to the geoaccumulation index (Igeo classification), on the other hand, most concentrations of selected trace metals in fish tissue were contained the highest concentration of most the detected heavy metals, while muscles appeared to be the last preferred site for the bioaccumulation of metals. This study showed that fish tissue contained high levels of heavy metals which are higher than the limits set by the FAOs. A recommendation is given to forbid fishing from that area of Lake Edku. The consideration of environmental factors is required to develop pollution managements and assess environmental risks for bed sediments.

RP096 Applying the Biochemistry of the "SOS" Mechanisms of Mercury Toxicity to Reduce Uncertainty of Metal Toxicity Estimates

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Methylmercury (MeHg) toxicity is characterized by: 1.) long latency of onset of symptoms following toxic exposure, 2.) neurological tissue specificity of pathological effects, 3.) inhibition of selenoenzymes in brain tissues, 4.) oxidative damage in the affected tissues, 5.) accentuated fetal vulnerability, and, 6.) preventative/rescue effects accompanying supplemental dietary selenium (Se) –commonly referred to as the "Se-protective" effect. These factors had been difficult to explain, but understanding has recently improved. Since Hg's affinity for selenium (Se) is ~1 million times greater than its affinity for sulfur, Se is now recognized as the molecular target of MeHg toxicity. MeHg inhibits synthesis and activities of Se-dependent enzymes that are required for the health of brain tissues. The 25 genetically distinct and functionally elite enzymes expressed in humans perform essential functions in brain physiology. The effects of high MeHg exposures arise from its unique abilities to irreversibly inhibit selenoenzyme activities. The involvement of Se-physiology in mechanisms of MeHg toxicity were not initially recognized because selenoenzyme metabolism and its unique roles in brain and endocrine tissues were generally unknown. However, in recent years, the Se-dependent aspects of their physiology are becoming better understood. Thus, the characteristic features of MeHg toxicity have become easy to understand. High MeHg exposures induce a sequence of biochemical reactions known as "SOS" mechanisms. These are; Synergies of Sequestration (SOS-1), Silencing of Selenoenzymes (SOS-2), Sequestration of Selenium (SOS-3), Suicide of Selenium-Deprived Cells (SOS-4) and Sustained Oblivion of Sec Synthesis (SOS-5). These disruptions have consequences that increase in severity as tissue MeHg concentrations approach, and especially as they exceed equimolar stoichiometry with tissue Se. The expected effects of "SOS" mechanisms coincide with observations in cell culture, animal models, and epidemiological studies while also clarifying features of MeHg toxicity which had previously been inexplicable. These advances appear likely to enhance computational modeling approaches for Hg and other metals in aquatic and terrestrial systems and will have immediate significance in environmental remediation and regulatory programs. They are the basis for development of the Health Benefit Value (HBV) criterion which is currently the only fully reliable MeHg risk assessment index.

RP097 CO2-induced acidification increases metal bioavailability and toxicity to marine organisms

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Sediments act as sink and source of anthropogenic chemicals such as metals to the water column. The chemical form in which metals are present in sediments will determine their mobilization capacity and bioavailability and, consequently, its potential toxic risk. However, changes in environmental conditions such as acidification might increase mobilization of metal from solid to liquid phase. This study investigates the effects of acidification caused by CO_2 leakage from carbon capture and storage activities on metal bioavailability and toxicity to marine organisms. A CO_2 bubbling system was designed in the laboratory for the conduction of a battery of tests to monitor the quality of sediments collected in areas with different levels of metals contamination. Various pH treatments were applied in the experiments. The results showed that, in general,

interactions between acidification and contamination increases the toxic responses of the species tested. For example, in experiments performed with fertilized females of the copepod *Nitokra sp.* fecundity tended to decrease with acidification. In addition, a smaller number of descendants was detected in the polluted areas when compared to the unpolluted one. This difference in progeny number could be related to the presence of metals in the sediment. Synergetic effect of acidification and metals was also observed in behavior response of the mussel *Mytella charruana*. In this sense, burrowing activity of the mussels was significantly affected when this specie was exposed to sediments containing high levels of metals. In all pH treatments tested, the proportion of animals buried in clean sediments was higher than in contaminated sediments, with the lower burrowing rate average (7% and 5%) in pHs 6.5 and 6.0 respectively at contaminated site that contrast to 14% and 23% clean site. Besides that, mortality data revealed that CO2-related acidification would lead to lethal effects on mussels, especially at the lowest pH analyzed (6.0), which presented the high mortality mean rate in both experiments (47% in clean site) with the highest rate in contaminated site (76%). These results suggest that the two stressors, acidification and metal contamination, act negatively to organism's health, especially in synergy. This study contribute to a better understanding about the implications of acidification caused by CO_2 leakages deriving from Carbon Capture and Storage technology in the behaviour of metals and its effects in the biota.

RP098 Comparison of metal toxicity and metabolic rate between northern and temperate fish species

J. Ford, Wilfrid Laurier University; J. McGeer, Wilfrid Laurier University / Biology

Canadian Water Quality guidelines (WQG) are developed from acute and chronic toxicity data, usually done using standard toxicity testing methods. Within the data sets used for deriving WQG, northern species are rarely represented and this represents a gap in our understanding as well as a concern. There is potential that metabolic rate (MR) could be used as an indicator in determining metal toxicity, due to its role in delaying toxicity. The objective of my research will be to determine if metabolic rates can be used as an indicator in determining toxic stress. MR, measured as the rate of oxygen consumption (MO_2), will be measured for rainbow trout and fathead minnows. Environmentally relevant concentrations of copper, including the LC50 and LC20 will be used to observe differences in MR during metal exposure between species. Lastly, similar tests will be done using northern caught slimy sculpin (*Cottus cognatus*) to determine if similar changes are seen. Preliminary results from one trial with added Cu (100 µg/L), control MO_2 ranged from 9.75 to 11.75 µmol/g/h (5 cycles, 11.05 ± 0.99 µmol/g/h, mean ± s.d.). When Cu was introduced, the MO_2 increased by 45% (3 cycles, approx. 1 h, 15.99 ± 0.88 µmol/g/h, mean ± s.d.). Subsequently, MO_2 decreased to 12.13 ± 0.36 µmol (mean ± s.d.). This study will be helpful in determining if water quality guidelines are relevant in the protection of northern species.

RP099 Implementation of an In Situ Pore Water Sampling Program for Metals to Obtain Closure at a Sediment Site

T. Caputi, Brown and Caldwell; T.L. Sorell, Brown and Caldwell / Risk and Toxicology; B. O'Neill, Brown and Caldwell; J. Rogers, Impact Environmental Closures, Inc.

An ecological risk assessment was completed to address sediments along the Delaware River shoreline adjacent to a former foundry site. The principal Chemicals of Potential Ecological Concern (COPEC) are metals. Results of bulk sediment samples collected from 12 stations adjacent to the site and submitted for both sediment toxicity and bulk analytical testing were inconclusive with regard to assessing ecological risk. While sediment toxicity testing using two species and three endpoints did not reveal any relationships between COPEC concentrations and toxicity results, some stations exhibited toxicity, and screening-level food chain modeling suggested the potential for toxicity through bioaccumulation. As a result, the New Jersey Department of Environmental Protection (NJDEP) requested additional confirmation that dissolved phase metals were not responsible for the observed toxicity. In response, a pore water sampling program was developed. Pore water sampling for metals is complicated by the tendency for metals to undergo solubilization upon exposure to oxygen, potentially overestimating the actual dissolved concentrations in the in situ pore water. To reduce the potential for bias, in situ pore water samplers were constructed and deployed. Customized samplers were constructed of Nalgene tubes equipped with 0.45-micron membranes under locking caps. Samplers were thoroughly deoxygenated with nitrogen prior to deployment at two depths (0-6 and 6-12 inches). After a month, samplers were removed and the contents submitted for metals analysis. A comparison of the results to ecologically based surface water quality standards indicated absence of dissolved phase metals at levels of concern and lack of unacceptable risk to native receptors in the study area. NJDEP concurred and no further investigation regarding ecological risk for sediments is required at the site.

RP100 Metal concentrations in biological specimens from wild and captive Hawaiian green sea turtles (Chelonia mydas)

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The Hawaiian subpopulation of green sea turtles is listed as threatened under the Endangered Species Act of 1973. Toxic heavy metals have been shown to decrease immune function, impair growth and decrease reproduction in wildlife. This study compares metal concentrations in green sea turtles raised in captivity at Sea Life Park on Oahu, Hawaii, to wild green sea turtles around the Main Hawaiian Islands. The turtles at Sea Life Park were brought into captivity in the mid-to-late 1960's. They represent one of the few captive breeding colonies of sea turtles in the world. They are fed a nutritionally complete, commercially available diet of pelleted food. Blood and scute (keratin comprising the turtle shell) samples from six green turtles at Sea Life Park and nine similarly sized wild green turtles were selected from the NIST Marine Environmental Specimen Bank. These samples, along with the pelleted food, were analyzed by Inductively Coupled Plasma - Mass Spectrometry (ICP-MS). Blood provides a good representation of recent exposure to metal contaminants, while scutes indicate long term exposure. Copper, Se, V, and Cr were determined to be higher in the scutes of captive turtles whereas As concentrations were higher in wild turtles. The metal concentrations seen in these Hawaiian green sea turtles are similar or orders of magnitude lower than concentrations seen in wild green sea turtles inhabiting coastal areas of Texas and California. There was no significant difference in metal concentrations between the scutes of male and female turtles at Sea Life Park. This is the first study to compare metal concentrations between captive and wild green sea turtles. Results from the forthcoming analysis of blood samples will also be presented.

RP101 Species and regional differences in chemical feeding ecology of mercury in Aleutian Island groundfish

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Monomethyl mercury ($MeHg^+$) accumulation in fish is both a human and wildlife health concern because it is a potent neurotoxin that crosses the blood-brain barrier. Mercury, mostly as $MeHg^+$, accumulates in fish through bioaccumulation and biomagnification, and can reach levels of concern in higher trophic feeding individuals. The environmental transport and accumulation of Hg in fish is driven by a variety of environmental factors including Hg source, types of transport mechanisms, methylation and demethylation activity, as well as fish age, size, and feeding ecology. The Aleutian Islands (Alaska) are host to one of the largest commercial fisheries in the country, as well as numerous rookeries of Steller sea lions (*Eumetopias jubatus*; SSL). Steller sea lions and Pacific halibut (*Hippoglossus stenolepis*) from the western portion of the Aleutian Islands have higher total Hg concentrations ([THg]) than SSL or halibut from other areas of Alaska. Numerous factors may be involved including long-range atmospheric deposition, seismic activity and geologic weathering, marine geothermal vents releasing Hg and/or altering methylation, or specific aspects of the feeding ecology for marine animals in the region. This project seeks to address regional and species driven components to these questions by investigating how region and feeding ecology of multiple fish species (sampled in the same time and space) are potentially driving [THg] in the food web. In collaboration with the fishing industry, we collected over 1,000 fish, representing 22 species (commercial target and bycatch species) from the central and western Aleutians. We analyzed muscle samples for [THg] and stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) to interpret how feeding ecology influences [THg]. Unadjusted [THg] ranged widely from 7.53 to 1578.26 ng/g ww. After length-standardizing [THg] for each fish, it is evident species and feeding ecology are more influential on [THg] than region or fish size. Thus, biomagnification and basic physiological differences between species are determined to be key drivers. Region had minimal influence on [THg]. Generally, [THg] increase with increasing trophic position and fish length in a species-dependent manner. Data presented here indicate that there is likely another environmental or ecological driver influencing the [THg] in SSL and halibut from the western part of the Aleutians that is independent of the fish assessed in this study.

Next Generation Nanotechnology – Environmental Health and Safety of Nano-enabled Products and Advanced Materials

RP103 Assessing release of engineered TiO2 nanomaterials in the weathering environment

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Titanium dioxide is a commonly used nanomaterial in consumer products[1]. Two desirable properties are that it is pure white and in some forms, is photoactive. Self-cleaning cement utilizes both properties, the cement is a pure white and stays clean due to photo-oxidization inducing the breakdown of organic contaminants. TiO_2-containing concrete samples were added to a group of test materials in an outdoor weathering network. The network exposes materials to natural weather conditions across a variety of climates in the United States. Locations include Tempe, AZ; Corvallis, OR; Golden, CO; Baltimore, MD; Vicksburg, MS; and Pittsburg, PA. The intent of the network is to study the role of climate on the release of nanomaterials from products. Samples are suspended in jars which passively collect rainwater that runs off the sample, while simultaneously exposing the sample to solar radiation and other weather conditions. Rainwater was collected monthly and analyzed for total titanium by ICPMS. Imagining techniques and single particle ICPMS were also used to characterize the form of titanium found in the jars, including control jars that did not contain test samples. Dust that deposited in the control jars contained significant amounts of titanium, which appeared to be particulate when analyzed by single particle ICPMS. Control jars in the first month of exposure, July 2016, had 5.2±0.2, 1.7±0.2, and 0.8±0.1 µg Ti in Tempe, AZ; Golden, CO; and Baltimore, MD; respectively. The cement sample containing jars had slightly more Ti on average with 5.3±0.1 ug Ti in Tempe, AZ; 2.6±0.3 µg Ti in Golden, CO, and 1.1± 0.1µg Ti in Baltimore, MD. The high background results in interference when determining the amount of engineered titania released from the concrete. The presentation will summarize the results of the one plus year of cement weathering at five locations as well as the development of approaches used to address the interference. [1]Haynes. V.N.; Ward, J.E.; Russell, B.J.; Agrious, A.G. *Aquatic Toxicology* 2017, *185*, 138-148

RP104 Altering silver nanowire dimensions to reduce toxicity and achieve safety by design

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Silver nanowires (AgNWs) are metallic, semiconducting nanomaterials that have been integrated into thin films, fabrics, and touchscreen display applications. AgNWs are expected to replace indium tin oxide (ITO) for touchscreen applications due to their relatively high transmission and low resistance properties. Despite an extensive effort to understand the human and environmental health implications of many engineered nanomaterials, including silver nanoparticles, the specific hazards of nanowires are not well understood. It is possible that AgNWs will exhibit altered transport and toxicity due to their large aspect ratios relative to spherical nanoparticles of the same composition. This project seeks to determine key links between the material properties of AgNWs, AgNW-enabled products, and their potential for inducing human and environmental toxicity. The goal of this work is to identify AgNW properties of concern and inform AgNW synthesis methods to reduce AgNW hazards to biological systems. AgNWs of various dimensions (length of 2 µm, 10 µm, and 25 µm; diameter of 30 nm, 60 nm, and 90 nm) were synthesized using the polyol process (a well-known synthesis method in the AgNW industry) and used for toxicity testing and examining AgNW release from AgNW films. Preliminary results indicate differential toxicity associated with AgNW diameter, and these results are consistent across multiple cell lines and cytotoxicity assays. Release scenarios indicate the extent of AgNW release from AgNW-enabled films is dependent on the characteristics of the film and the type of mechanical or chemical stress that is applied. Future work will include whole organism toxicity assays using rainbow trout fry, exploring potential mechanisms of toxicity with RNA seq, investigating the materials properties of AgNWs relative to their altered dimensions, and further examination of AgNW release from embedded matrices.

RP105 The impacts of graphene oxide and silver-graphene oxide on freshwater microbial communities

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Graphene oxide (GO) and silver-functionalized GO (Ag-GO) are applied in various fields such as biotechnology, medical science, and environmental engineering due to unique material properties such as hydrophilicity, high surface area, and mechanical strength of GO and antimicrobial and antiviral properties of silver. As the usage of such nanomaterials increases, they could enter the aquatic environment during production, product use, and disposal. However, the effects of GO and Ag-GO on aquatic microbial communities are not well known. Therefore, we determined the impacts of GO and Ag-GO on the aquatic microorganisms inhabiting a river and a lake located in Seoul, Korea. Unfiltered natural water samples were exposed to GO and Ag-GO with a final concentration of 0 to 100 mg L^{-1}. During a 3-day exposure test, microbial responses were determined by analysing extracellular enzyme activity and nitrification rate. Also, the release of lactate dehydrogenase and generation of intracellular reactive oxygen species were analyzed to determine how GO and Ag-GO alter the microbial activities. The activity of leucine aminopeptidase was decreased to 8 % and 4 % by 100 mg GO L^{-1} and 100 mg Ag-GO L^{-1}, respectively, compared to the control (0 mg GO or Ag-GO L^{-1}, enzyme activity of 100 %). Also, the nitrification activity was significantly inhibited by the GO and Ag-GO treatment. 5 % of intracellular lactate dehydrogenase was released under 100 mg GO L^{-1} and 4% under 100 mg Ag-GO L^{-1} treatment when compared to the control (0 %), indicating the cell membrane damage. Also 80 % of intracellular reactive oxygen species was generated under 100 mg GO L^{-1} and 37 % under Ag-GO L^{-1} treatment when compared to the control (0%). The activities of microorganisms inhabiting natural surface waters can be inhibited by oxidative stress and cell membrane damage induced by GO and Ag-GO. Our results can contribute to the development of systematic regulations on emerging engineered nanomaterials.

RP106 Microbial toxicity of Ga2O3, In2O3, GaAs, and InAs nanoparticles in semiconductor manufacturing effluents

C.H. Nguyen, J.A. Field, R. Sierra-Alvarez, University of Arizona / Chemical and Environmental Engineering

In the State of Arizona, semiconductor manufacturing is an important industrial sector which requires significant amounts of water for processing. One of the processes with the highest water demand is chemical mechanical planarization (CMP), a step used to polish wafer surfaces into ultra-flat surfaces through a combination of physical and chemical forces. The CMP process utilizes slurries of abrasive nano-sized particles (NPs) such as alumina (Al_2O_3), ceria (CeO_2), and silica (SiO_2). Annually, millions of gallons of CMP waste are discharged to municipal sewers. Because III-V materials such as gallium arsenide (GaAs), indium arsenide (InAs), and gallium indium arsenide (GaInAs) can provide high electron mobility with wide and adjustable band gaps and reduce power consumption, the semiconductor manufacturing sector is planning to introduce III-V film structures into silicon wafers to enhance device performance. Aqueous solutions generated during the planarization of III-V films are expected to contain a mixture of metal oxides NPs as well as indium (In), gallium (Ga), and arsenic (As) species. The presence of toxic III-V species and NPs in CMP effluents is a concern. While the aquatic chemistry and ecotoxicity of arsenic species have been investigated extensively, essential information is needed for the environmental chemistry and ecotoxicity of other III-V species. Consequently, research is required to understand the speciation, adsorption, transport, and toxicity of III-V materials in natural water and engineered systems. This presentation will discuss the results of preliminary experiments conducted to investigate the microbial toxicity of nano-sized gallium oxide (Ga_2O_3), indium oxide (In_2O_3), GaAs, and InAs. These materials can potentially be formed during polishing operations of GaInAs films. Different tests were performed including the widely used commercial toxicity Microtox bioassay (inhibition of bioluminescence) as well as bioassays using microbial populations important in wastewater treatment, specifically, anaerobic methanogenic microorganisms. The results obtained indicated that Ga_2O_3 and In_2O_3 NPs arenot inhibitory at concentrations as high as 500 mg/L. In contrast, the GaAs and InAs NPs caused 50% methanogenic inhibition at concentrations of 5.7 and 129 mg/L, respectively. Additional experiments demonstrated that the main mechanism of GaAs and InAs toxicity is dissolution and release of toxic arsenic oxyanions (chiefly trivalent arsenite).

RP107 Measuring nanomaterial transformations and biouptake on a single particle and single cell basis

J. Lead, University of South Carolina / Center for Environmental NanoScience and Risk / Arnold School of Public Health

Improved metrology for nanomaterial transformations in complex media and for the quantification of biouptake and accumulation is urgently required. Developing a better understanding of nanomaterial environmental fate and behavior and biological effects is hampered by a current knowledge gap in this area. Here we present the development of single cell inductively coupled plasma mass spectrometry (SC-ICP-MS), and the application of this and single particle ICP-MS alongside core-shell nanomaterials. SP-ICP-MS and the nanohybrids have shown the concentration-dependence behavior of the nanomaterials, with an aggregation-dominated regime at concentrations > 10 ppb and a dissolution dominated regime below 1 ppb. SC-ICP-MS has been applied to algal biouptake studies with high but species/size specific uptake rates and amounts. The SC-ICP-MS, with cell counting techniques, allowed discrimination between cells containing 0, 1, 2 and 3 nanoparticles per cell. Combination of detailed transformations and uptake studies has allowed improved understanding of exposure, dose and bioavailability.

RP108 Reproductive and Developmental Impacts of Next Generation Battery-Related LiNiMnCoO2 and LiCoO2 Nanoparticles on D. melanogaster, C. riparius

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Due to its highly tractable, fully-mapped genome, high percentage of disease genes homologous to humans, and a short life cycle of approximately 10 days, *Drosophila melanogaster* is used as a model organism. *Chironomus riparius*, a freshwater midge important to the aquatic food chain was also examined as a model organism. Both species were used to examine the potential toxic effects of exposure to $LiNiMnCoO_2$ (NMC) and $LiCoO_2$ (LCO), which are targeted as next-generation battery-related nanoparticles. The current work is the first to examine the impacts of complex metal oxides LCO and NMC to these species. Existing investigations of the potential impacts of nanoparticles have largely focused on model or simple nanomaterials, and previously published nanotoxicology studies utilizing *D. melanogaster* commonly exposelarvae to nanoparticles via mixture with food media, which introduces additional variables that may affect outcomes. The work presented here describes a minimal media exposure method in which *D. melanogaster* embryos hatch directly into treatment media consisting of only nanoparticles and moderately hard water. *C. riparius* larvae were exposed in aqueous environments with sand substrate. In initial trials, no significant mortality was observed in *D. melanogaster* larvae exposed to NMC nanoparticles at 1, 10, 20 or 100 mg/L, utilizing two different methods: nanoparticle-treated food media and nanoparticle-treated aqueous suspension. Subsequent aqueous exposures to LCO and NMC showed impacts on the fecundity of *D. melanogaster* – although not necessarily on a dose-dependent basis – and significant impacts to development of *C. riparius* at concentrations of 10 and 100 mg/L of both NMC and LCO in terms of larval growth and emergence to adult imago phase.

Ecotoxicology and Environmental Health in the Developing World

RP109 Investigating insecticides in water and sediment of the Choapa River, Chile: Do they sink or swim?

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In Chile, freshwater rivers travel short distances from the Andes Mountains to the Pacific Ocean. The areas immediately adjacent to the rivers are used to produce fruits and vegetables, and it is possible that these areas can contribute insecticides and herbicides to the riverine systems during rainstorm runoff events. The paucity of knowledge about the occurrence and environmental fate of pesticides in these novel systems presents a unique challenge for ecotoxicology. In the Choapa River, we have found that a native pencil catfish, the (*Trichomycterus areolatus*) exhibits alterations in gene expression. The alterations are occurring in genes that are biomarkers for endocrine function as well as oxidative stress. Given the biological evidence, we sought to determine 1) whether pesticides detected in the Choapa River were consistent with the changes observed in the fish biomarkers, and 2) whether these agrichemicals were predominately found in surface waters or sediment. To address this objective, we collected water and sediment samples from 5 different sites along the Choapa River, then analyzed for a suite of 27 common herbicides, insecticides and their metabolites. Water and sediment samples were prepared onsite then analyzed at the Water Science Lab, University of Nebraska Lincoln, using gas chromatography–mass spectrometry (GC-MS). Preliminary analysis detected the herbicides propazine and metribuzin, as well as the insecticide carbofuran, all of which varied seasonally and spatially within the Choapa River. Finding from our chemical analysis and observations of seasonal and El Nino trends reveal a complex interplay between agrichemical occurrence and river discharge in the Choapa Basin. Additionally, the results of this study illustrate the challenges of analytical environmental chemistry in unique environments, and the importance of appropriate sample schemes of water and sediment.

RP110 Assessing risk of exposure to heavy metals by consumers of fish samples from Donga River Nigeria

O. Otitoju, Federal University Wukari / Biochemistry; G.T. Otitoju, University of Nigeria Nsukka / Home Science Nutrition and Dietetics; R. Boyi, Federal University Wukari / Biochemistry

In recent time, the safety of fish which has long serve as cheap source of animal protein and other essential nutrients to humans have been threatened. This is due to the increase in anthropogenic activities particularly industrial/mining activities and agricultural practices. In this study, the levels of the heavy metals (Pb, As, Cd, Hg, Zn, Cu, Cr, and Fe) river Donga, Taraba State, Nigeria were assessed using fish samples as a case study. Five fish species (*Synodotis membranaceus cupside*, *Protopterus annectens*, *Clarias gariepinus*, *Heterotis niloticus* and *Tilapia zilli*) were collected for this analysis. The result obtained indicated that with exception of Pb all other heavy metals (As, Cd, Cr, Cu, Fe, Hg, and Zn) are present in concentrations above the permissible limits as well as the provisional tolerable week intake (PTWI) (Cd: 0.007mg/kg, As: 0.015mg/kg, Hg: 0.004-0.0016mg/kg, Pb: 0.025mg/kg) and provisional maximum tolerable daily intake (PMTDI)

RP111 Dioxin levels in breastmilk of Vietnamese residents living on or near Agent Orange/dioxin contaminated and reference sites

D. Pham, H.L. Bergman, University of Wyoming / Zoology and Physiology; R. Nielson, Western Ecosystems Technology, Inc.

Agent Orange was the main defoliant used by the US military in Vietnam from 1961-1971; this herbicide was contaminated with dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin, or TCDD). There are several identified major dioxin ‘hot spots’ resulting from Agent Orange use, distribution and spillage at former US military bases in Vietnam. Recent studies have found high TCDD concentrations in all media exceeding international standards. Elevated dioxin concentrations in the food chain are likely sources of human dietary exposure, posing a very serious health risk for the Vietnamese population living on or near these hot spots. In animal studies, exposure to TCDD could disturb the physiological processes leading to endometriotic progression and alteration of spermatogenesis. Additionally, studies by Manikkam et al. (2012) suggest that dioxin exposure in gestating F0 generation females could promote epigenetic transgenerational inheritance of adult-onset diseases in subsequent generations. Acknowledging the potential effects of dioxin exposure and our recent published research on risk factors associated with dioxin accumulation in Vietnamese residents (Pham et al. 2015), our objective for this study is to review the trend of TCDD concentrations in breast milk samples of Vietnamese women at contaminated and reference sites. This study also compares published dioxin breast milk levels in Vietnamese women and other study sites around the world.

RP112 A Novel Non-Selective Passive Sampling Device to Measure Over 500 Organic Chemicals in the Lancang-Mekong Watershed

W. Wang, Yunnan University / Asian International Rivers Center; X. Xia, X. Kong, D. Shea, North Carolina State University

The use of hydrophobic passive sampling devices (PSDs) such as semipermeable membrane devices (SPMDs) and low-density polyethylene (LDPE) have proven very useful for measuring longer-term (e.g., 30-day) time-weighted average (TWA) exposure to hydrophobic chemicals with log K_{OW} values above about 4.5. However, making similar TWA measurements for more polar chemicals with log K_{OW} below 4.5 have proven problematic. A polar organic chemical integrative sampler (POCIS) was developed for more hydrophilic organic contaminants (log < 3.0), however the POCIS does not provide reliable quantitative exposure estimates. And there remains a gap between what SPMDs and POCIS can accumulate with chemicals in the log K_{OW} range of about 3.0 to 4.5 not accumulating reproducibly in either device. We have developed a more universal non-selective passive sampling device (nsPSD), which is composed of a non-selective porous stainless steel cartridge and a mixed-polymer sequestration sorbent. The nsPSD has been calibrated in the laboratory for over 500 organic chemicals with log K_{ow} values ranging from 0.3-8.15. These chemicals include current-use pesticides, organochlorine pesticides, polychlorinated biphenyls, polycyclic aromatic compounds, pharmaceutical and personal care chemicals, natural algal toxins, and many metabolites and other degradation products of these chemicals. We have deployed these samplers at numerous field sites and in most cases the TWA exposure estimates using the nsPSDs closely match those using sequential grab sampling (every 1-2 days) over the time of deployment. We report here on the laboratory calibration and field verification of this new nsPSD using data from the Lancang-Mekong River. We believe this new nsPSD will have very broad application to most organic chemicals and has the potential to offer a significant advance in decreasing the uncertainty in risk assessment by increasing our capability to estimate chronic exposure of chemicals in water.

Wildlife and Terrestrial Toxicology

RP113 Agglomeration of Escherichia coli with positively charged nanoparticles can lead to artifacts in a standard Caenorhabditis elegans toxicity assay

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The increased use and incorporation of engineered nanoparticles (ENPs) in consumer products requires a robust assessment of their potential environmental implications. However, a lack of standardized methods for nanotoxicity testing has yielded results that are sometimes contradictory. Standard ecotoxicity assays may work appropriately for some ENPs with minimal modifications, but produce artefactual results for others. Therefore, understanding the robustness of assays for a range of ENPs is critical. In this study, we tested silicon, polystyrene, and gold ENPs with different charged coatings and sizes to examine the robustness of a standard *Caenorhabditis elegans* toxicity assay. Of all of the ENPs tested, only those with a positively charged coating had any toxic effect on growth of *C. elegans* using the standard toxicity assay. The positively charged ENPs were observed to heteroagglomerate with *Escherichia coli* cells, impacting the ability of nematodes to feed, and leading to a false positive toxic effect on *C. elegans* growth and reproduction. We then tested these ENPs in two alternate *C. elegans* assays that did not contain *E. coli* to further investigate their potential ecotoxicity and found greatly reduced toxicity of ENPs at the concentrations tested.

RP115 Bioaccumulation of Polycyclic Aromatic Hydrocarbons from Microplastics by Earthworm Eisenia fetida

J. Wang, University of California Riverside; J. Gan, University of California, Riverside / Environmental Sciences

Microplastics (MPs) are widespread in the environment, which have been shown to sorb chemical pollutant from their surrounding environment, thus raising concern as to their role in the movement of these pollutants through the food chain. Compared to the ocean, the soil may also receive MPs from different sources and through different pathways. However, few results on the effects MPs in soil on terrestrial fauna has been published. This study investigated the role of microplastic as a carrier of hydrophobic organic contaminants (HOCs) in soil. Different types of plastics with similar diameter (250 – 350 µm) were employed, including High-Density Polyethylene, Low-Density Polyethylene, Polystyrene, PolyVinyl Chloride, and Polypropylene. For the first experiment, earthworms were exposed to MPs with sorbed PAHs in clean soil. The results demonstrated that MPs could be ingested by earthworm, and PAHs derived from MPs could be assimilated into the tissues of earthworm. For the second experiment, earthworms were exposed to PAHs-contaminated soil with clean MPs. The results indicated that MPs could reduce PAHs uptake compared to the control. Disposable solid phase microextraction fibers were also used to measure the free dissolved concentrations (Cfree) in soil. The results of Cfree were consistent with the bioaccumulation. Although MPs in the environment may lower PAHs uptake compared to unabsorbed free chemicals, our results have demonstrated MPs can transfer PAHs into the terrestrial organisms. Therefore, MPs may cause a potential risk of terrestrial food chains through dietary sources. This study demonstrated that MPs can act as a vector for the assimilation of HOCs into terrestrial organisms.

RP116 Biochemical Alterations due to PCBs and PBDEs Exposures in Cats

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Polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) are among the most harmful organic pollutants. Because of their structures and chemical properties, they can distribute in environment, accumulate through food chains, and affect aquatic animals, human, and wildlife health. These organic compounds generally disrupt endocrine systems, particularly thyroid homeostasis. Feline hyperthyroidism related to increased PCB and PBDE levels in cat serum have been reported worldwide, while the effects of PCBs and PBDEs exposures on serum biochemical as well as liver and kidney functions in cats are not known. The purpose of this study was to evaluated serum biochemical including liver enzymes, albumin, total protein, blood urea nitrogen (BUN), creatinine, and lipids by using the collected time-series serum from cats (*Felis catus*) exposed to PCBs (short-term single exposure; i.p.) and BDE209 (one-year oral administration). In PCBs exposure cats, the albumin and total protein levels were significantly decreased at one time by comparing with control cats. The significant negative correlation between total OH-PCBs and BUN (R=-0.42, $P< 0.05$), creatinine (R=-0.39, $P< 0.05$), and triglycerides (R=-0.40, $P< 0.05$) were also found in PCBs exposure cats. Whereas the significant decreasing albumin level and the increasing high-density lipoprotein (HDL) and triglyceride levels were noted in BDE209 exposure cats at one time or another. In addition, the negative correlation between BDE209 and creatinine (R=-0.50, $P< 0.05$) were obsereved in BDE209 exposure cats. Even though there are no signs of liver cell damage, which was suggested by no significant difference of liver enzymes between control and exposure cats of both experiments. However, these results indicated that both PCBs and BDE209 exposures can affect liver and kidney functions, as well as disturb lipid metabolism. These negative correlations revealed that OH-PCBs and BDE209 are key affectors for these biochemical alterations, and mechanism of actions should be verified by further biomolecular studies.

RP117 Effects of in ovo exposure to two persistent organic pollutants, SCCPs and TBBPA-BDBPE, on thyroid-related gene expression in American kestrels

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Highly brominated flame retardants are being replaced by alternative FRs such as Tetrabromobisphenol A bis[2,3-dibromopropyl ether] (TBBPA-BDBPE). TBBPA-BDBPE was introduced as a possible substitute for decabromodiphenyl ether (decaBDE), but has shown similar persistency and environmental transport mechanisms. This additive flame retardant is used in plastic products, resins, textiles, paints, and household electronics. Although it is produced only in the US, Israel, and China, TBBPA-BDBPE is detected in environmental samples and wildlife tissues from across the globe. Short-Chain Chlorinated Paraffins (SCCPs) are priority emerging persistent organic pollutants (POPs) identified as chemicals of concern by the Stockholm Convention, Environment and Climate Change Canada, and the U.S. Environmental Protection Agency. SCCPs are used in metal lubricants and coolants in metal cutting, and as plasticizers and flame retardants in plastics and paints. SCCPs are of concern because they bioaccumulate in wildlife and humans, are environmentally persistent, transported globally, and are toxic to aquatic organisms at low concentrations. However, few data are available on the potential adverse effects of TBBPA-BDBPE and SCCPs in birds. A comparative exposure assessment of these two classes of flame retardants was conducted using egg injections in a non-model species, the American kestrel (*Falco sparverius*) to assess survival, molecular, biochemical, and endocrine, growth and reproductive endpoints. Although we recently identified potential effects of these POPs on thyroid gland histology and thyroid hormone levels in these birds, liver transcriptome analysis using RNAseq did not identify any significant effects on thyroid-related gene expression in a limited number of samples. Therefore, to clarify the discrepancy between these results, we used the transcriptome to develop primers for a pre-selected set of thyroid-hormone related genes and analyzed their expression in a larger number of liver samples using qPCR. Preliminary results will be presented.

RP118 Estimation of avian dermal exposure within current regulatory exposure modeling for ecological risk assessments

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Understanding dermal exposure in relation to total exposure is important for higher-tier ecological risk assessments of crop protection chemicals to birds. Historically, generation of dermal absorption and dermal toxicity data have focused on mammals (i.e., rat and mouse) as a surrogate for human exposure with data for birds, reptiles, and amphibians generally absent. Despite this lack of data, understanding the contribution of dermal exposure is an important component for higher-tier risk models such as EPA's Terrestrial Investigation Model (TIM) that predict avian exposure from multiple routes. The dermal component of TIM utilizes a ratio of oral to dermal LD50's called F_{red} (i.e., oral LD50 / dermal LD50) as a multiplicative factor for estimating exposure via the dermal pathway. However, for many crop protection chemicals avian dermal data are not available and a modeled dermal LD50 value must be estimated from avian oral toxicity data. Within the TIM model, dermal LD50s are estimated using a regression equation of avian dermal and oral LD50 data for organophosphate and carbamate insecticides. Dermal LD50s for all compounds are solved using oral LD50 data and the slope and intercept from this regression equation. The inflection point where this equation crosses the 1:1 line is approximately the log oral LD50 = 2.21. Thus for oral LD50s greater than the inflection point, dermal LD50s are always lower resulting in a multiplicative effect of F_{red} on the overall dermal exposure estimate. For relatively non-toxic compounds, this approach results in calculated dermal LD50s that may be significantly biased low (i.e., toward a much greater toxicity than the oral LD50) and resulting in an inflated dermal exposure estimate, often several times greater than the fractional dose attributed to food ingestion. We discuss the magnitude of effect this TIM model anomaly has on dermal exposure estimates, potential alternative approaches, how dermal absorption data could be used to correct this anomaly, and the implications of the current model approach on higher-tier risk assessments.

RP119 Evaluation of Monotonicity of Concentration Response in Avian Reproduction Studies

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The Avian Reproduction Test (OECD 206 and OPPTS/OCSPP 850.2300) is required in several geographies for registration of pesticide active ingredients. The objective of the study is to evaluate the effects of dietary exposure to consistent concentrations of the test material over a period of >20 weeks and determine a no observed effect concentration (NOEC). Variables measured evaluate impacts on parental health, reproduction, and offspring viability. With consideration of the high resource demands of the study design and effort to reduce vertebrate animal use, most studies are performed with the minimum criteria of 3 treatment groups and a control with 12 to 18 male-female pairs/treatment. Typically, the most powerful statistical tests to determine concentrations of test substances that cause observable effects are based on the assumption of a monotonic response to increasing concentration. However, the limited number of treatment levels in a typical avian reproduction study does not permit assessing the change in sign of the slope of a dose-response relationship within the range of doses examined (i.e., a commonly accepted definition of non-monotonicity). Due to a lack of additional guidance or alternative approaches/definitions for determining monotonicity, stakeholders assessing the data may evaluate the data twice: once assuming a monotonic response and then again assuming a non-monotonic response, after which the lowest NOEC value is selected. Alternately, NOEC decisions may be based on "visual interpretation" of the data, irrespective of the statistical robustness of the endpoint. This project will generate an anonymized database of registrant avian reproduction toxicology data from bobwhite quail and mallard duck studies. Monte-Carlo type simulations will be used to generate simulated dose response curves with known degrees of response and monotonicity and where responses have random distributions derived from the compiled database. Competing sets of rules for defining monotonicity will be developed and applied to the simulated responses to evaluate the ability of the rules to optimize statistical power of tests used to detect effects. After the best performing rules are selected, they will be applied to the original data set to determine the proportion of studies that are classified as non-monotone. CropLife America envisions that these analyses will provide useful information for the evaluation of monotonicity in avian reproduction studies.

RP120 Individual and combined acute toxicity of metribuzin, imidacloprid and benomyl on earthworm, Eisenia fetida

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Pesticides have become widespread pollutants of soils due to their large applications in agriculture and homes. An earthworm assay was used to assess the acute toxicity of metribuzin, imidacloprid and benomyl with different modes of action. Ecotoxicities of these pesticides were compared for earthworm Eisenia fetida separately and in combination in artificial soil test. Results from 14-days soil toxicity test showed a different pattern of toxicity except that imidacloprid was the most toxic under the soil toxicity bioassay system. The ternary mixture of metribuzin–imidacloprid–benomyl displayed a significant synergistic effect on the earthworms under the soil toxicity bioassay. Understanding of the mechanisms of combined pesticides effects on non-target organisms would valuable for purposes of environmental risk assessment.

RP121 Mammalian Ecological Soil Screening Value Refinement for Antimony

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Antimony (Sb) is used in a number of industrial applications, including as a flame retardant, pigment and alloy-hardening material, and is commonly detected in soil at contaminated sites. For Sb, the U.S. Environmental Protection Agency's ecological soil screening level (Eco-SSL) for protection of mammalian wildlife is lower than naturally occurring background concentrations of antimony in soil. As such, the Eco-SSL does not accomplish the objective of distinguishing soils where Sb clearly poses no risk from soils where Sb requires further evaluation. At the time the Eco-SSL was developed, data were unavailable to calculate realistic soil to invertebrate tissue bioaccumulation factors (BAFs), and therefore, a conservative default BAF of 1 was used in the absence of better information. We conducted a comprehensive review of recent (2005 – present) literature documenting uptake of antimony into earthworm tissue from soil and derived a more realistic BAF. Our review focused on laboratory or field exposed soil invertebrates that were depurated prior to total metal analysis. Soil and worm antimony concentrations were compiled from 5 studies. From this data set, we identified individual study mean BAFs ranging from 0.06 to 0.81, and an overall average BAF of 0.36. We also examined the mammalian toxicity reference value (TRV) underlying the Eco-SSL and identified the estimated water ingestion rate for rodents in the critical reproductive toxicity study as a potential source of over-conservatism. Substituting a water ingestion rate specific to laboratory rodents and utilizing a more refined interpretation of reported body weight results in a slight increase in the estimated no-effect TRV. Taken together, these adjustments increase the Eco-SSL, but the value is still less than typical background soil concentrations. If data were available to estimate Sb bioaccessibility in worms ingested by mammals, a more realistic screening value could be developed. We also examine the nature and magnitude of Sb effects at the lowest observed adverse effect level for reproduction, to aid interpretation of Sb exposures in excess of the no-effect TRV. Overall, there is an obvious data gap with respect to relevant mammalian toxicity endpoints for antimony.

RP123 Mercury Concentrations in Terrestrial and Aquatic Primary Consumers in Tennessee's Appalachian Mountains

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The Appalachian Mountains are typically thought of as pristine, but due to their high elevation and location, they are susceptible to atmospheric deposition of non-point source contaminants such as mercury. Mercury is traditionally thought of as an aquatic problem because once it reaches the aquatic environment, sulfate-reducing bacteria may convert mercury into its bioavailable form, methylmercury. Previous research has shown that methylmercury can bioaccumulate in primary consumers and biomagnify through aquatic and terrestrial food webs. The objective of this study was to determine if terrestrial and aquatic primary consumers have comparable mercury concentrations.To determine this, an aquatic primary consumer (caddisflies, Order: Trichoptera) was collected at four sites spanning the latitudinal gradient of Tennessee's Appalachian Mountains. Additionally, a terrestrial primary consumer was sampled concurrently; millipedes (Class: Diplopoda) were collected at three sites and terrestrial snails, (Class: Gastropoda) were collected at one site. Whole-body homogenates were analyzed for total mercury concentrations, and subsamples were analyzed for methyl-mercury. Results show that total mercury concentrations in millipedes (470±110 µg/kg) and snails (16.2±5.66 µg/kg) exceeded total mercury in aquatic primary consumers (3.3±.18 µg/kg). Currently, no explanation can be given on why millipede mercury concentrations were over an order of magnitude greater than the aquatic primary consumer used in this study.

RP124 Metal Accumulation in Terrestrial Animals

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Metals are commonly used in many facets of modern society and are thus frequently released into the environment. Metal accumulation in wildlife may occur as a result of excess metals in the environment, which in turn can lead to toxicity when concentrations exceed certain threshold levels. Unfortunately, tissue metal concentrations in many terrestrial mammals are to a large extent unknown. Here, we propose the first analysis of liver metal concentrations in many wildlife species collected from sites in Georgia and Florida that experienced relatively similar levels of human disturbance. Liver tissues were obtained from hundreds of mammals (armadillos, bobcats, coyotes, feral cats, opossums and raccoons) and were screened from multiple sites and for multiple metals (copper, zinc, nickel, cadmium, lead, and silver). Differences in metal concentrations due to age (juvenile versus adult), sex, and time (yearly variation) were examined. This study will provide the first extensive reference data for metal concentrations in various key wildlife species found in terrestrial habitats. Results of this study will also increase understanding of metal accumulation and transfer in terrestrial food webs.

RP125 Mining legacy features as attractive nuisances: The November 2016 waterfowl mortality event at the Berkeley Pit, Butte, MT

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The Berkeley Pit, part of the Silver Bow Creek/Butte Area Superfund Site in Butte, Montana, was created by open pit mining for the removal of copper ore. Nearly 1.5 billion tons of ore and waste rock was removed creating a pit nearly 1,780 feet below grade. When mining ceased, groundwater infiltrated the pit and currently is over 1,000 feet deep and covers over 300 surface acres. This water is acidic with pH ranging from 2.37 to 3.41, and contains high concentrations of metals including copper, cadmium and zinc. In November 2016, after a mild fall, tens of thousands of snow geese (*Chen caerulescens*) and Ross's geese (*Chen rossii)* flew towards Butte where they encountered winter storm conditions that disrupted migration. Thousands of these birds landed in the Berkeley pit and attempts to haze the birds off the pit were only partially successful. Over the next week, three to four thousand gees died. The U.S. Fish and Wildlife Service collected a subset of dead snow geese from the pit and submitted them to the U.S. Geological Survey-National Wildlife Health Center. Full necropsy was conducted on four snow geese and the cause of death was suggested to be acute topical damage to oral, gastrointestinal, or respiratory mucosa. Common findings among these birds included acute upper gastrointestinal tract and duodenal mucosal necrosis, upper respiratory tract mucosal necrosis, peracute cutaneous erosions and ulcerations of the unfeathered portions of legs and feet, and acute renal tubular necrosis. Tissue toxic elements panel on liver and kidney of three of the four prosected snow geese demonstrated elevated levels of manganese and zinc in both organs compared to reference levels for domestic geese. Tissue copper, iron, and cadmium levels were elevated in these organs, but not consistently by organ or goose. The analytical and necropsy results from this recent event are similar to the results from a smaller snow goose mortality event documented in 1995 on the Berkeley Pit, as well as laboratory studies exposing mallards to synthetic acid metalliferous water. New hazing strategies are currently in development in an effort to prevent future mortality events.

RP126 Next Steps for Refining Terrestrial Vertebrate Risk Assessments of Pesticide Treated Seeds

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The Environmental Fate and Effects Division (EFED) of the Environmental Protection Agency (EPA) in the United States routinely conducts ecological risk assessments (ERAs) for seed treatment pesticides. In March of 2016, EFED issued a memorandum ("Refinements for Risk Assessment of Pesticide Treated Seeds – Interim Guidance") to clarify current EFED policy and ensure that ERAs for pesticide-treated seeds are consistently conducted using appropriate risk assessment methods. The memo also outlined potential refinement options. The long-term goal is to incorporate the information into subsequent versions of the Terrestrial Residue EXposure (T-REX) tool. For seed treatments, T-REX currently calculates two risk quotients (RQs) for birds and mammals: 1) a dietary-based RQ (Acute #1) and 2) a LD_{50}/ft^2 RQ (Acute #2). As with the risk assessment approach for granular pesticides, incorporation rate (IR) is used to indicate seed availability on the soil surface in the Acute #2 scenario. IR is a function of the prescribed planting method on the pesticide label. However, Acute #1 and Chronic exposure scenarios rely on the concentration of active ingredient on the treated seed, and thus cannot be refined using application methods that reduce seed availability on the soil surface. Two refinement options are suggested for Acute #1 and Chronic scenarios where RQs exceed established levels-of-concern (LOCs): 1) foraged area of concern, and 2) foraged time to concern (for birds only). The first refinement determines whether sufficient seeds are available within their home range to cause effects given the foraging range of birds. The second refinement determines whether sufficient foraging time exists for birds to collect enough seeds to reach the LOC in a single day. This project will evaluate the refinement options proposed in the 2016 EFED memo. Building upon these proposed refinements, we will highlight other areas where refinements could be added to T-REX. Examples include using actual diets of bird species in agroecosystems at times when the pesticide may be used, incorporation of foraging behavior on and off treated fields, accounting for changes in pesticide loads on seeds over time, and including temporal availability of planted seeds. Our goal is to stimulate discussion of refinement options for avian assessments of seed treatments in future versions of T-REX.

RP127 Novel comparison of toxicity of hydrophobic and hydrophilic quantum dots and their ligands using C. elegans

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A knowledge gap exists for toxicity of hydrophobic nanomaterials, likely because exposure to cells or many models organisms is not feasible for materials that are not water-soluble. Quantum dots (QDs) are being manufactured for use in modern displays and are being researched for use in photovoltaics and as imaging agents. Investigations into toxicity and molecular mechanisms of interaction of quantum dots (QDs) with cells and organisms have up to now focused largely on hydrophilic variants of these fluorescent nanomaterials - those proposed for use as imaging agents. However, the major type of QDs manufactured for use in displays and photovoltaics are hydrophobic, covered with trioctylphosphine (TOP) and trioctylphosphine oxide (TOPO) ligands. Hydrophobic QDs used in displays are generally CdSe/ZnS QDs, and toxicity from these heavy-metal-containing QDs is assumed to come from Cd ions released by the particles. Investigations of hydrophilic CdSe/ZnS quantum dots have not found these water-soluble QDs to be acutely toxic, and they are less toxic than an equivalent mass of Cd. Unlike many models, the nematode *C. elegans* is amenable to exposure to hydrophobic materials, as this extensively characterized and manipulable model can be cultured on solid media. In addition, this 1 mm soil worm is transparent, making tracking of fluorescent particles such as QDs inside the organism possible. Thus, *C. elegans* is ideally suited for investigating the toxicity and interactions of hydrophobic QDs with a biological system. This study compares CdSe/ZnS QDs functionalized with hydrophobic TOP/TOPO ligands to identical QDs functionalized with hydrophilic DHLA-PEG ligands, and compares toxicity observed to comparable amounts of each respective ligand and Cd. Fluorescence imaging for hydrophobic QD-exposed nematodes was also carried out to visualize interactions of hydrophobic QDs with the organism. Results suggest that acute toxicity observed for exposure to hydrophobic QDs is caused by TOP/TOPO ligands, likely due to the corrosive nature of these ligands to tissues. Based on these results, investigations are underway to determine if less-toxic hydrophobic ligands can be substituted for TOP/TOPO to mitigate impacts of hydrophobic particle exposure.

RP128 Short-term induced molecular stress responses in coelomocytes of Eisenia fetida earthworms exposed to silver nanoparticles

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In recent years the production of a great variety of products containing nanoparticles (NPs) has increased massively. The subsequent release of NPs into the environment has created a need to assess the potential ecological risk in soil, water and air. Silver nanoparticles (Ag-NPs) have the highest degree of commercialization due to their high thermal and electric conductivity, high catalytic activity, and powerful antimicrobial properties. *Eisenia fetida* is a model specie in soil toxicity studies and has been broadly used due to its sensitivity to different toxicants at different levels of biological organization. The main aim of the present investigation was to understand the effects produced by AgNPs (5.08±2 nm sized and PVP-PEI coated) in comparison with the soluble form of the metal ($AgNO_3$) at molecular level in coelomocytes of *E. fetida* at different exposure times. *E. fetida* were in vivo exposed to different concentrations of Ag-NPs and $AgNO_3$ (0.05 and 50 mg Ag/kg soil) through OECD artificial soil for 1, 3 and 14 d. Then, the transcription levels of selected genes associated to oxidative stress (Catalase) and metal detoxification (MTs-metallothioneins) were determined in coelomocytes extruded from exposed earthworms. In addition, the enzymatic activity (Catalase) and protein content (MTs) were quantified. The responses varied significantly among days, exposure concentration and Ag form. Exposure to Ag-NPs led to a significant induction of CAT at day 1, followed by an increase in its transcription levels after 3 and 14 d of exposure. Similarly, exposure to $AgNO_3$ induced the transcription of CAT at day 1 but at day 14 a down-regulation was observed. The CAT activity increased at both treatment and exposure times (1 and 3 d). After 14 d of exposure, CAT activity was inhibited at the highest concentration tested. The highest increase of MTs at protein level was observed after 3 d of exposure. Our results indicate that short-term exposures to Ag-NPs induced early molecular stress responses (MT induction and oxidative stress) in coelomocytes that precede other responses at higher levels of biological organization. The responses in translational level in *E. fetida* tissues were according. The study indicates the importance of using integrative biomarkers for the evaluation of the potential risk of Ag-NPs in soils.

RP129 Spatial and temporal trends of trace metals and polychlorinated biphenyl congeners in tree rings from western Kentucky

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Pine trees are used as biological indicators to assess environmental contamination by relating contaminant concentration in annual growth rings. The objective of the present study was to understand the spatial variation and temporal trends of inorganic elements including copper, manganese, chromium, lead, vanadium, nickel, zinc, arsenic, silver, cadmium, indium, tin and organic compounds viz. PCB congener concentrations in tree rings of pine trees from Western Kentucky. Pine tree core samples were collected from selected locations including industrial, national parks and undeveloped areas. Standard operating procedures were followed for tree core sampling and analysis using ICP-MS and GC-ECD. The results revealed that several elements and lower chlorinated PCB congeners were found in annual growth rings of pine trees. Tendency for increasing trends of chromium, copper, zinc and slight declining trends for lead and some PCB congeners were observed.

RP130 Concentration-Dependent Inhibition of Acetylcholinesterase by Organophosphate poisoning in dogs: A Biochemical and Electrographic study

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Organophosphate poisoning (OP) is one of the most common poisonings in developing countries. In this study, twenty-four dogs in four groups of six each were used. Control group bathed with water only, group B with 16% Coumaphos (recommended), groups C and D with times 10 and 20 of 16% Coumaphos, respectively. Blood was collected from cephalic vein for biochemical assays. Electrocardiographic parameters were assessed from a Lead-II electrocardiogram. There was a significant increase ($p< 0.05$) in total cholesterol in group B and D compared to the control. LDL-cholesterol decreased significantly ($p< 0.05$) in all groups compared to the control. The activity of superoxide dismutase (SOD) reduced ($p< 0.05$) significantly across all the groups and even after 36 hours of exposure. However, the activity of the glutathione peroxidase (GPx) was not affected following exposure to OP. The serum reduced glutathione (GSH) fell in a concentration dependent manner in all animals exposed to OP. Coumaphos exposure led to a significant ($p< 0.05$) increase in serum MDA in a concentration dependent manner after 36 hours post exposure. The serum nitric oxide (NO) and MPO content increased ($p< 0.05$) significantly following exposure to different concentrations of Coumaphos. The activity of Acetyl cholinesterase (AchE) fell significantly from the normal concentration of the OP down to the highest concentration. The activity of serum creatine phosphokinase (CK) increased ($p< 0.05$) significantly in groups C and D compared to the control and recommended concentration. Electrocardiographic abnormalities recorded included low-voltage R-waves, first degree heart block, significant increased ($p< 0.05$) heart rate (HR) and shortened QT interval compared to the control and recommended concentrations. Taking together, coumaphos poisoning caused an inhibition of AchE and significant potentially fatal arrhythmias via the induction of oxidative stress.

Wildlife Ecotoxicology – Linking Exposure to Effect Cascades

RP131 Anticoagulant Rodenticides and Wildlife: Synthesis

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Anticoagulant rodenticides (ARs) are the cornerstone of rodent control throughout the world. A new reference text prepared by 24 scientists addresses AR use, regulation, exposure pathways, toxicity, mechanism of action, pathology, pharmacokinetics, genetic resistance, non-target risk and its mitigation, pesticide alternatives and integrated pest management (IPM). Key concepts include: blood clotting is a highly conserved process, and thus AR use to control rodents must be balanced against potential non-target species effects; development of genetic resistance in pest species led to use of potent long-lived second-generation ARs; vitamin K_1 can be an effective antidote (feature absent for many other rodenticides); pharmacokinetic, ecological and behavioral processes account for differences in non-target sensitivity; field monitoring studies indicate >50% of predatory wildlife contain AR residues; while AR residues indicate exposure, a definitive role for ARs in mortality requires additional evidence (e.g., clinical signs, hemorrhagic lesions); probability of death in relation to AR residues may help assess the extent of mortality in populations; tissue residues are informative of exposure, but dietary AR concentrations are more suited to assess risk; and primary AR exposure associated with urban, agricultural and island conservation use may cause localized non-target species population declines, but there is no clear evidence for secondary AR exposure causing population declines. While ARs pose significant risk to non-target wildlife, their authorized use in many countries demonstrates that benefits outweigh risks. Alternatives to ARs (e.g., bromethalin, cholecalciferol, zinc phosphide) also pose a significant risk to non-target wildlife, livestock, companion animals and people. Information needs include: better understanding of AR-induced effects and risk to wildlife populations; knowledge of exposure and effects in invertebrates and lower vertebrates; efficacy of ARs on pest species control and management of resistance; and development of safe alternative chemical and non-chemical methods. Such data will help address fundamental questions like "How much rodent control is warranted?", "What are the consequences of such activities on non-target wildlife?" and "How effective are AR risk mitigation measures (e.g., pulsed baiting, limiting AR use to professional applicators) and IPM in controlling pest species and minimizing non-target effects?"

RP132 Heavy metals in blood and feathers of migratory raptors

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Identifying the location of contaminants that migratory birds encounter along their annual cycle is a long-time question among ornithologists and ecotoxicologists. The Gulf of Mexico's coastal plain of Veracruz is a primary corridor for migrating raptors headed to their austral, non-breeding sites. Our research aims to answer a single question: Where do migratory birds acquire more metal contaminants during their annual migration cycle? We hypothesized that sampling heavy metals in blood and feathers would help us identify the boreal or austral source of contamination. We collected blood and feather samples from juvenile (hatch year) *Accipiter cooperii* and *Accipiter striatus* during the migratory passage in the autumn of 2016, as well as reference samples from a resident species, *Falco femoralis,* in San Isidro, Veracruz, Mexico. Each of the seventy-five blood samples and its corresponding feather samples were analyzed with a Metrohm voltammetry system for zinc, cadmium, lead, mercury, and copper. We compared the differences of the median values among species and also made orthogonal, pairwise comparisons using one-way Kruskal-Wallis tests. The concentrations of heavy metals in feathers was 1-2 orders of magnitude larger than in blood for all species. In order of importance, the median zinc levels in feathers were (all ww ppm): *A. striatus* at 1649.859, *A. cooperii* 564.134, and *F. femoralis* 1094.658 ppm, with highly significant differences among species (P = < 0.001). Cadmium in feathers median values for *A. striatus* 15.208, *A. cooperii* 15.139, and *F. femoralis* 12.356, with n.s. differences. Lead median values for *A. striatus* was 253.543, *A. cooperii* 85.425, and *F. femoralis* 133.815, significantly different among species. All other tests in blood and feathers were low, with no statistically significant differences among species. Feathers provided substantially different concentrations of heavy metals compared with blood. The different concentrations of Zn and Pb in hard tissue (feathers) of migrants versus control species reveal contaminant source in breeding, boreal sites. Contaminants in blood, in turn, indicate short-term exposure: our current results indicate that metal exposure in Veracruz is not significantly different than that of their breeding sites.

RP133 Quantifying the effects of 17ß-trenbolone exposure on Japanese quail egg yolk production using a double-dye technique

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Vitellogenin is a precursor egg yolk protein and its production has been directly linked to egg quality and number in fish; however this relationship has not been tested in birds. Current efforts are underway to model avian vitellogenesis in order to link impairments of vitellogenin production to reductions in fecundity following contaminant exposure. Therefore, to test if vitellogenin can be used as an indicator of avian fecundity we experimented with a double-dye technique to quantify daily egg yolk production in Japanese quail. In this experiment, twelve male and female Japanese quail pairs were exposed for three weeks to either 0 or 20 ppm 17ß-trenbolone, an anabolic steroid previously shown to inhibit egg production in Japanese quail. Two colors of fat-soluble dyes were fed on alternate days to the females via gavage. Once ingested, the dyes are transferred and envelope the developing ova. Eggs were collected when laid, processed and analyzed for yolk production. The yolks contained rings of dye which allowed for an estimate of daily yolk deposition using circumference measurements of each ring. Following exposure, plasma was collected from the females and analyzed for sex steroid concentrations and total zinc (a commonly used surrogate for plasma vitellogenin). Exposure to 17β-trenbolone reduced the number of rings present however yolk volume within an individual egg was similar between treatments. Additionally, vitellogenin, egg production and total yolk deposition were reduced following the 17β-trenbolone exposure suggesting that an impairment of vitellogenin production following contaminant exposure can lead to egg production losses in birds.

RP134 Avian early-life stage chemical exposure test: A prospective tool in ecological risk assessment

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Currently, there is no internationally-accepted, standardized protocol for early-life stage chemical exposure in avian species. This represents an important gap to fill in terms of providing an alternative approach to live animal tests for chemical screening and prioritization. Several labs use egg injection approaches to administer priority chemicals to developing avian embryos. In this study, we compared the effects of benzo[a]pyrene

(BaP) and chlorpyrifos (CPF) exposure between a laboratory model species, Japanese quail (JQ), and a wild avian species of environmental relevance, double-crested cormorant (DCCO), using a standard egg injection procedure developed in house. BaP and CPF were injected into the air cell of un-incubated, fertilized eggs of the two species at 3 concentrations (plus a solvent control group) and sampled at two distinct developmental time points: 1) mid-incubation (day 9 and 14 for JQ and DCCO); and 2) 1-2 days prior to hatch (day 16 and 26 for JQ and DCCO). Liver tissue was collected at mid-incubation for subsequent 'omics analyses (i.e. transcriptomics, proteomics, metabolomics) and chemical residue analysis, while apical endpoints (e.g. development, growth, deformities) were assessed in late-stage embryos. BaP exposure led to a 50% reduction in viability at 0.5 µg/g in JQ and CPF reduced JQ embryo growth and caused severe malformations at 40 µg/g. Data are currently being further analyzed to link 'omics endpoints with apical measures of regulatory relevance and those from the DCCO egg injection study are pending. By employing this strategy, we aim to anchor apical endpoints to molecular and biochemical changes that occur early in development, in accordance with the 21st century shift in risk assessment, which promotes a mechanistic rather than whole animal approach. These studies complement a larger set of planned exposures (additional 6 chemicals in JQ and 2 in DCCO) to support the standardization of this early-life stage test in Canada and a large-scale Genome Canada project (EcoToxChip project; @ecotoxchip) aimed at transforming ecological risk assessment.

RP135 Evaluation of Sample Preservation and RNA Extraction Methods for Transcriptomic Analysis of Avian Blood Collected in the Field

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Transcriptomic applications in environmental toxicology for the purpose of measuring adverse effects on gene expression in wildlife are well established. Such methodologies have targeted tissues such as liver, kidneys, brain, thyroid, and gonads, among others. Limited research has been published on the application of transcriptomic methods to blood, largely because blood has a large extracellular component, and additionally mammalian erythrocytes are anucleate. On the other hand, avian erythrocytes are nucleated, thereby offering an opportunity for this matrix to be analyzed as a non-invasive transcriptomic endpoint. Standard preservation methods for transcriptomics include the use of preservative solutions such as RNALater or rapid freezing using liquid nitrogen. Collection of samples in the field, however, presents unique difficulties because access to liquid nitrogen may be limited, and previously published reports suggest that the use of RNALater for blood collection, at least in mammals, does not provide high quality RNA. Therefore, we tested several methods of blood preservation and RNA extraction to identify the combination that produces the highest quantity and integrity of total RNA (tRNA) from whole avian blood. Method development considered sample preservation (i.e. frozen RNAlater-preserved blood vs refrigerated RNAlater-preserved blood vs unpreserved flash-frozen whole blood vs archived hematocrit fraction), as well as various extraction protocols. Japanese quail (*Coturnix coturnix*), American kestrel (*Falco sparvarius*), and bald eagle (*Haliaeetus leucocephalus*) blood was tested for this analysis. Results were analyzed by Nanodrop spectrophotometry, electrophoresis, and on a Bioanalyzer to provide data on RNA quantity and quality. These methods can be applied to field and laboratory settings for transcriptomic assessment of this biological matrix in avian species.

RP136 Marine mammals as sentinel species for monitoring persistent organic pollutants (POPs) in marine ecosystem

Y. Jeong, B. Gu, K. Park, J. Lim, Hanyang University; H. Moon, Hanyang University / Marine Sciences and Convergent Technology

Marine mammals have been utilized as sentinel species for chemical pollutiton in marine ecosystem due to their long life span, high trophic level, and large body burden. Exposure of POPs is crucial problem due to their adverse health effects to the present and next generations. Therefore, as sentinel, measurement and estimation of POPs using marine mammal are important for monitoring ocean health. For the meaningful monitoring program of POPs using marine mammals, several criteria such as selection of specimen and biological tissues should be clarified. In this study, we analyzed eight groups of legacy and emerging POPs (PCBs, DDTs, HCHs, CHLs, HCB, PBDEs, PFCs, and HBCDs) in major organ tissues including blubber, liver, kidney, stomach, melon, and muscle from long-beaked common dolphins (n=6) and finless porpoises (n=8) with different sex and growth stages collected from Korean coastal waters during 2012-2015. We purposed to suggest suitable specimen and organ tissues for measuring each POPs group. Organ tissues from finless porpoise showed higher concentrations of PBDEs and PFCs while PCBs and organochlorine pesticides (OCPs) (e.g., DDTs) showed comparable or lower concentrations than those measured in tissues from common dolphins. This result seems to be due to the different exposure sources (e.g., diet and habitat) and metabolism. In both species, mature male showed the highest POP concentration due to their age- and sex-dependent (delivery and lactation) accumulation, except for PFCs. Congener profiles of PCBs and PBDEs in mature female of common dolphin were clearly different with those found in immature specimens and mature males, indicating the re-distribution of POPs through lactation and delivery. In all of the analyzed organs, DDTs showed the highest concentration due to their widespread contamination and persistence despite the regulation. Among organ tissues, the highest POP concentrations were found in blubber and melon associated with high levels of lipid. PFCs were higher in liver and kidney due to their protein-binding properties. Regression analysis of POPs between blubber and other organ tissues was significant for almost all of the chemical groups except PBDEs. This suggests that blubber is well-reflected matrix to predict the POP levels in other organ tissues. Our results suggest that marine mammal can be used as sentinel of POPs in marine ecosystem and as substitute to estimate body distribution of POPs in mammalian species.

Quality Assurance and Control of Chemical and Toxicological Measurements

PC001 Bringing quality to an unregulated academic environment

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Most academic research includes little to no QC and QA because there is no legal requirement and the benefits are not immediate. Although the advantages of quality management are several (better science, less risk, stronger funding proposals), it is not easy to implement on a small scale. A lack of infrastructure, resources, knowledge, and guidance can slow progress even if buy-in has been achieved. While true GLP compliance may be unrealistic for small labs, the basics of a quality management system can be designed in to a new research project or program. We have voluntarily done this for a basic lab research project in wildlife toxicology. The major elements, challenges, benefits, and lessons learned will be discussed along with suggestions on how to do this more effectively and more commonly. For example, establishing a change control system and creating SOPs are straightforward, while making effective use of an independent QA unit requires thought. We also briefly discuss development and validation of a bioanalytical method for measuring perfluorinated substances in amphibian tissues.

PC002 What's the bottom line? – Part 2: The Continuing Story

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Advances in analytical instrumentation have not only increased the number and types of chemicals measured, but reduced the quantitation limits, allowing these chemicals to be detected at progressively lower concentrations in various environmental matrices. Such analytical advancements have been a particular benefit to the measurement of pharmaceuticals, hormones, personal care products and other contaminants of emerging concern (CECs). Nevertheless, even as specific methods for CECs are being developed, concentrations of many CECs are still near or below the threshold of these improved detection limits. The complexity of these analyses is further compounded by the fact that many CECs are so pervasive in our surroundings that they may contaminate samples during collection, processing, or analysis. To ensure confidence in data being generated, an integrated suite of quality assurance (QA) procedures and quality control (QC) samples are necessary. These QA procedures and QC samples prevent or address potential for contamination or loss in both the field and the laboratory, and discriminate between field or laboratory introduction of these artifacts. These QC samples include, but are not limited to, field and laboratory blanks, replicate samples, matrix fortified samples, fortified laboratory samples and instrument blanks. This presentation will examine the QA protocols implemented and QC sample results from a recent study of surface water, wastewater effluent and drinking water jointly conducted by the USEPA and the USGS. Not surprisingly, matrix enhancement was more pronounced in the effluent samples than in the surface or drinking water samples. Detections above the instrumental detection limit but below the reporting level were measured in all three sample types, with relatively more of these low levels of detections in the surface water samples. The presentation will examine how the handling of detections hovering near the threshold of detection influences the data set.

PC003 Flexibility of NIST Natural Matrix Reference Materials for Benchmarking Environmental Measurements

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The National Institute of Standards and Technology (NIST) has a long history of creating natural matrix reference materials for use in benchmarking measurements for trace elements, organochlorine pesticides, polychlorinated biphenyl congeners, and polycyclic aromatic hydrocarbons. The reference materials initially produced by NIST were assigned measurement values based on the current needs of the environmental measurement community. However, these needs continually evolve as information on new chemical occurrence comes to light. The result is a requirement for reference values for new chemicals in natural matrix reference materials. NIST has repurposed many existing natural matrix Standard Reference Materials (SRM) by adding new values for chemicals based on emerging needs or emerging technologies. For example, Standards Reference Material (SRM) 2585 Organic Contaminants in House Dust, is an existing material that is currently being updated for perfluorinated alkyl substances, phosphorous flame retardants, and nitro musk compounds. Since its release, SRM 1947 Lake Michigan Fish Tissue has had values added for perfluorinated alkyl substances, fatty acids, brominated flame retardants, and mercury isotopes. Other examples of evolving SRMs include SRM 1944 New York New Jersey Waterway Sediment (brominated flame retardants, polychlorinated napthalenes), SRM 1946 Lake Superior Fish Tissue (perfluorinated alkyl substances), SRM 1945 Organics in Whale Blubber (brominated compounds) and SRM 2781 Domestic Sludge (perfluoroalkyl substances, brominated flame retardants).

Solutions for Conducting Experiments With Difficult Substances

PC004 Can passive dosing be the alternative to carrier solvent or water-soluble fraction method in testing poorly water-soluble substances?

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When poorly water-soluble substances are subjected to aquatic toxicity test, the difficulty on achieving/maintaining required exposure concentrations should be taken in account. To overcome these problems, passive dosing methods were investigated and applied for various aquatic toxicity tests. In passive dosing, the test chemicals are loaded to polymers (e.g. polydimethylsiloxane, PDMS). Then, loaded polymer is placed into test chamber, working as a "partitioning donor" which makes possible to achieve and maintain the target concentration. In this study, medical grade PDMS was applied for OECD acute toxicity tests such as *Daphnia sp.,* Acute Immobilisation Test of poorly water-soluble substances (e.g. polyaromatic hydrocarbons: PAHs, and organohalogen compounds). Additionally, the results were compared with test solutions prepared by conventional methods (using carrier solvents and "water-soluble fractions" or WSF) in testing poorly water-soluble substances. When a four-ring PAH pyrene was used as a test chemical, passive dosing could successfully achieve five exposure concentrations, including water-soluble concentration. Additionally, 85~97% of the initial concentrations were maintained after 48 h, while the concentrations decreased to 51~70% in the tests with carrier solvent and WSF. Although the initial exposure concentrations were similar between the passive dosing and WSF, the EC_{50} value of WSF was significantly low. EC_{50} values by passive dosing and carrier solvent methods were rather comparable; however, the different dose-response patterns (i.e. slope) were demonstrated. In the passive dosing system, *D.magna* neonates were trapped by the surface of PDMS placed at the bottom of the test vessels, even in the control condition. Along with the modification of preparation method of test vessels, the method's extent of applications remained to be investigated further (e.g. vessel volume, test period). This study will provide case studies of passive dosing with the other poorly water-soluble chemicals to identify the technical issues of passive dosing in the application to standardized ecotoxicity tests.

PC006 Investigation of alternative morphological endpoints during UVCB exposure to zebrafish

B. Hedgpeth, ExxonMobil Biomedical Sciences Inc.; J. Butler, ExxonMobil Biomedical Sciences Inc. / Environmental Toxicology and Chemistry Laboratory; A.D. Redman, Exxon Mobil Biomedical Sciences / Toxicology and Environment Science Division; R. Alyea, M. Lampi, ExxonMobil Biomedical Sciences Inc.

Danio rerio (zebrafish) embryos were used to investigate alternative sublethal morphological alterations when exposed to a range of seven petroleum substances of unknown or variable composition, complex reaction products and biological materials (UVCBs). Traditional acute embryo testing relies on four endpoints defined by the OECD 236 Fish Embryo Acute Toxicity Test guideline to describe exposure related effects (coagulation, lack of somite formation, tail-bud detachment from yolk-sac, and presence of heartbeat). Zebrafish embryos were exposed to seven UVCBs beginning at < 90 min post fertilization and observed for development over 96-120 hours. As detailed analytical measurements are challenging for UVCBs, biomimetic solid phase microextraction (BE-SPME) was used as the dose metric to quantify bioavailable exposure levels. Alternate endpoints consisted of cardiac dysfunction, pericardial edema, yolk sac edema, tail curvature, hatch success, pericardial edema area (μm^2), craniofacial malformation, swim bladder development, fin development and heart rate as well as the four observations established in OECD 236. At the conclusion of experimentation observations were combined to note commonalities amongst varying UVCB exposures. The most prevalent endpoints observed were pericardial and yolk sac edema and tail curvature. We were able to identify a BE-SPME threshold range in which morphological alterations preceded mortality. BE-SPME thresholds identified for alternative endpoints relate closely to acute/chronic thresholds developed in previous work. Further, this work aids in the development and understanding of interactions amongst UVCBs and developing zebrafish beyond traditional OECD 236 testing through the use of alternative morphological endpoints.

PC008 Design and Conduct of Aquatic Toxicity Tests with Difficult Test Materials

A. Samel, DuPont; A.J. Jones, DuPont Crop Protection / Institute of Environmental Toxicology; T. Scown, DuPont Crop Protection; H.O. Krueger, EAG Laboratories / Aquatic, Plant and Insect Toxicology

Test material with certain physicochemical characteristics can affect exposure concentrations in aquatic systems and make it especially challenging to design and conduct regulatory aquatic toxicity tests. For core regulatory aquatic toxicity tests, exposure concentrations not maintained within a certain range or with precipitate present are often rejected by regulatory reviewers. Test material properties that can impact the maintenance of test concentration levels throughout the test include volatility, sorption potential, hydrolytic or photolytic instability, and very low solubility in dilution test media. Because of the requirement to maintain constant exposure levels to the test organisms, study design is critical from preparation of test solutions, to test solution delivery to test chambers, to analytical confirmation of test solutions. The stability of the test material will determine if the test design will be a static, static-renewal, or flow through test system and test material solubility will determine the highest test concentration for the test. If a solvent is needed, the solubility and stability data for the test material in the organic solvent is necessary. If the test material sorbs to surfaces, the test system will need to reach equilibrium before the test can be begin and water samples for analytical confirmation may need to include organic solvent to mitigate any potential for the test material to adsorb to the sides of the analytical sample vials. Volatile test material will require specially designed test chambers to minimize head space where test material could partition. The purpose of the presentation is to discuss measures to consider when designing tests for difficult to test compounds.

PC009 Passive dosing of hydrophobic organic mixtures

R. Hammershøj, H. Birch, P. Mayer, Technical University of Denmark / Environmental Engineering

Regulatory biodegradation and toxicity testing is often conducted on single test substances, whereas in the environment chemicals are found as complex mixtures. Lately, test methodologies for complex mixtures of chemicals, such as multi-constituent substances and UVCBs (substance of Unknown or Variable composition, Complex reaction products or Biological materials) have received increased attention. However, testing hydrophobic organic mixtures is currently a challenge. Recent developments in passive dosing allow biodegradation and toxicity testing of composed mixtures controlled in terms of concentration and composition. This study focuses on expanding the application of passive dosing to complex mixtures, and more specifically to UVCB substances. The procedure is simple: a silicone rod is loaded with a liquid UVCB by direct swelling and subsequently equilibrated with water to create stable and reproducible concentrations of each mixture constituent. The concentration level is controlled by varying the amount of chemical added to the rod. This method enables creation of low concentrations for biodegradation and toxicity testing under realistic exposure conditions. The poster will focus on (1) the fast and reproducible loading of 21 UVCB mixtures: 9 petroleum products and 12 essential oils and (2) the optimized passive dosing kinetics for some selected UVCB mixtures.

PC010 The use of saturator columns in aquatic toxicology studies with a poorly soluble substance

A.J. Jones, DuPont Crop Protection / Institute of Environmental Toxicology; A. Samel, DuPont; T. Scown, DuPont Crop Protection; H.O. Krueger, EAG Laboratories / Aquatic, Plant and Insect Toxicology; D. Brougher, EAG Laboratories; B.T. Minderhout, J. Claude, EAG, Inc.

In order to demonstrate regulatory safety, aquatic toxicologists are often challenged by the need to assess the acute and chronic toxicity of poorly soluble substances in surface waters. It is challenging to test up to the water solubility limit of such chemicals using conventional mixing techniques (*e.g.* stirring, sonication, solvent addition, etc.) Difficulties may arise that include precipitation, suspensions, solvent toxicity, and unforeseen chemical and physical reactions with the test material. The use of saturator columns has been proposed as a means of achieving desirable test concentrations whilst avoiding the complications associated with other mixing methods. This presentation will compare the effective dissolution of a non-polar plant protection product using conventional approaches and saturator columns at multiple temperatures in multiple matrices, and will discuss the advantages and disadvantages of using saturator columns for regulatory testing. Results indicate that the use of saturator columns can increase test material concentrations in solution by 3-4 fold across temperature ranges and matrices while minimizing the negative effects of conventional dissolution techniques. This suggests that the use of saturator columns can be useful in situations where concentrations near the limit of solubility are required for toxicity testing.

PC011 Near real-time analysis of oil derived PAH by an antibody-based biosensor: a new tool to evaluate fate and transport

M.A. Unger, K. Prossner, G.G. Vadas, M. Vogelbein, Virginia Institute of Marine Science / Aquatic Health Sciences

The regular analysis for hydrocarbon contaminants in aquatic systems is a routine part of environmental analysis within the oil and gas industry. Examples of drivers are regulatory requirements, assessing remediation efforts or to monitor chemical fate/transport after an accidental spill. During spill events in particular, precise and rapid detection of hydrocarbons in the environment is essential for assessing fate/transport to inform accurate risk assessments. The standard analytical methodologies (e.g. GC-MS) are not conducive to rapid field assessments, are costly and time consuming. These hurdles can be prohibitive for assessing large numbers of samples and/or obtaining results in timely manner. As such, decisions on managing chemicals in the environment may be delayed as stakeholders wait for data. Conversely, immunochemical detection methods using antibodies have been increasingly gaining in acceptance. Here we evaluated a novel antibody-based biosensor (Li et al, 2016) with high specificity to 3 – 5 ring polycyclic aromatic hydrocarbons (PAHs) to demonstrate the biosensor's utility as a possible means to quantifying aqueous PAH during an oil spill. The biosensor was first tested via analysis of PAH in the Water Accommodated Fraction (WAF) of weathered and fresh oils. Measured concentration data from the biosensor matched the predicted concentrations of 3 – 5 ring PAHs based on fate models and the careful quantitative analysis of the neat oils. The technique was also compared to SPME analyses of WAF produced by various oil dosing techniques including CROSERF methods. Additional testing was performed at Ohmsett- The National Oil Spill Response Research & Renewable Energy Test Facility wave tank (Leonardo, New Jersey) to evaluate the biosensor's utility to detect oil plume migration. Spill scenarios were conducted with and without dispersant. Data from the antibody biosensor were compared to both passive samplers and more traditional spectrophotometric methods. The advantages and limitations of this near real-time analysis will be discussed. The potential for near real-time monitoring of hydrocarbon plume fate and transport will greatly assist spill response managers in assessing how to best direct clean-up and recovery operations.

The Other 3 Rs: Remediation, Rehabilitation, Restoration - When Do You Get a Passing Grade?

PC012 Embracing values for sustainable solution of a wicked problem: Restoration of contaminated sites

D. Larson, University of Minnesota / Northern Prairie Wildlife Research Center; A.M. Starfield, University of Minnesota / Ecology, Evolution and Behavior

Contaminant remediation, while not necessarily easy, is often seen as a straightforward (tame) problem. The contaminant must be removed or sequestered to reduce risk to humans or other receptors. In many instances, however, removal of threat is insufficient and restoration of ecosystem functions is desired. The potential for wickedness increases as stakeholders with different values weigh in on their perception of the problem. Presumably, all will agree that remediation is necessary, but disagreement may center on the pathway toward remediation and the desired future condition of the site. The problem becomes truly wicked when no one solution is "correct" and will satisfy all stakeholders. Here we suggest decision tools and heuristics to work through stakeholder engagement, problem formulation, and goal setting. At minimum, the problem should be formulated in terms of the three pillars of sustainability: environmental, social, and economic effects of both remediation and restoration. To do this, stakeholders representing each pillar will need to be involved in problem formulation, but without the formulation in hand, identification of stakeholders is itself a problem and their selection can strongly bias outcomes. A combination of snowball sampling – in which successive rounds of stakeholders suggest additional stakeholders – and rapid prototyping to update problem definition as stakeholders are added can demonstrate when adding more stakeholders is no longer significantly influencing the scope of the problem. At this point, goals for the restoration need to be established by both the recruited stakeholders and the core team (those responsible for legal and technical aspects of remediation). Teams of stakeholders with diverse interests should be assembled to develop goals around each pillar of sustainability. We recommend using the SMART criteria: each goal must be Specific, Measurable, Agreed-upon, Realistic, and Time-constrained. We then discuss how various techniques (such as scoring systems, Bayesian Belief Networks, decision trees) can be used to focus attention on goals and reach a good solution. This is an iterative process: as monitoring data allow increased confidence in model parameters, goals may need to be reassessed.

PC013 Sudbury, Canada - Lessons Learned from a Lifetime of Healing and Creating a Novel Functional Ecosystem on a Smelter-Impacted Landscape

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By the 1970's a hundred years of industrial activity of nickel and copper mining and smelting had resulted in 17 500 ha of land within regional watersheds that were devoid of vegetation and another 64 000 ha of semi-disturbed woodland. The internationally acclaimed Regreening Program commenced in 1978, directed by a citizen municipal advisory panel (VETAC). The original 'Sudbury Recipe employed was: 1) dolomitic liming (10 t/ha), fertiliser (400 kg/ha of 5:20:20) and sowing of an agronomic grass - legume nurse crop (40 kg/ha). Subsequently, 2) major tree species native to the regional forest were planted, often by community volunteers. Approximately 3450 ha have received stage 1 treatment and nearly 10 million trees and shrubs have been planted since 1979. Following an ecological risk assessment of Sudbury in 2002 - 2009 the risk management plan developed as the Sudbury Biodiversity Action Plan. Additional native trees and shrubs (>65 species) are now used along with modifications to sow mainly native grasses as the Modified Sudbury Recipe/Protocol. In addition there is deliberate transplanting of forest floor mats to promote expansion of understory species. The developing Sudbury ecosystem has become a mixture of the planted trees and shrubs, in a matrix of natural invaders with over 100 species of herbs, grasses, mosses and lichens contributing to the plant diversity. Plant growth in this novel ecosystem is similar to control areas outside Sudbury while metal content of the vegetation is only slightly elevated. The outcome of the Regreening Program is a new image for the city and environs which has helped to attract new business enterprises, tourists and encouraged an increased respect and use of the environment. Currently restoration completion criteria are under development.

PC014 Habitat Enhancement in a PCB Contaminated Floodplain - When is Habitat Enhancement an Option?

A.F. Brady, M.H. Henning, Ramboll Environ; J.M. Johnson, Bridgestone Americas

This case study describes development of remedial objectives, planning, implementation, and monitoring for a PCB-contaminated forested floodplain in Indiana. Early identification of remedial objectives provided a framework for remediation and restoration planning. Remedial options were considered to address two localized areas of PCB contamination in the undeveloped floodplain. Stakeholder engagement activities allowed input from local residents to be considered in development of the remedial alternatives. Residents desired preservation of the trees within the forested floodplain and minimal disruption of the community. The remedy selected for the undeveloped floodplain was habitat enhancement with focused vegetative stabilization. This remedy included establishment of groundcover in two areas with elevated PCB soil concentrations to stabilize and minimize contact with the soil, and installation of bat houses and tree seedlings to provide short-term and long-term bat habitat. Challenges associated with both implementation and monitoring of the habitat enhancement measures are described. An adaptive management approach was developed during implementation and monitoring that allowed for successful completion of the habitat enhancement monitoring program in 2016.

PC015 Balancing ecosystem characterization intensity with level-of-effort to design efficient restoration monitoring of Riparian hardwood forests

M.J. Hooper, M.A. Struckhoff, J.L. Albers, K. Grabner, N.S. Green, B.K. Kunz, USGS / Columbia Environmental Research Center; V. McDonald, University of Central Arkansas / Biological Science; S.E. Fetters, USFWS / Northeast Indiana Habitat Restoration Office; J. Isanhart, US Department of the Interior / Office of Restoration and Damage Assessment; D.W. Sparks, USFWS / Indiana Ecological Services Field Office; K. Skrabis, US Department of the Interior / Office of Policy Analysis

Highly visible and extensive restorations often include support for detailed monitoring programs that inform the public, trustees, and sponsors of progress. However, restoration monitoring can be expensive and is often the first thing cut when funding levels are less than desired or anticipated. Further, support for small sites with limited restoration activity precludes many standard monitoring approaches. These scenarios demonstrate the need for monitoring approaches ranging from comprehensive ecological assessments to more limited studies for restricted budgets and small sites. The Department of the Interior's Natural Resource Damage Assessment and Restoration program restores trust resources injured due to oil spills and hazardous substance releases. Restorations are funded through settlements or litigation with responsible parties, with settlements ranging from tens of thousands to multi-billion dollars, and covering sites of several acres to areas incorporating multiple states. Monitoring approaches must be sufficiently flexible to accommodate these ranges of restoration activity and level-of-support. To address this need, monitoring studies on three restored Midwestern riparian hardwood forests use a breadth of techniques with varying levels-of-effort designed to measure ecological constituents. The goal is to determine how much monitoring is necessary to provide sufficient knowledge to make informed progress assessments and management decisions compared to those made based on more intensive assessment techniques. Study methods range from simple and inexpensive (annual photo-documentation) to thorough and comprehensive (extensive replication across numerous sample plots), demonstrating information value for site managers and restoration practitioners through the range of cost:benefit options. Beyond ecological characterization, the different data sets will be assessed for their suitability for translation to ecosystem services and their sufficiency for recommending management options.

PC016 Using Adaptive Management to Move toward a Desired Restoration Trajectory: Providing Examples with Positive Outcomes

A. Farag, D. Larson, USGS; J. Stauber, CSIRO Land and Water; R.G. Stahl, E. I. du Pont de Nemours and Company / Corp Remediation; J. Isanhart, US Department of the Interior / Office of Restoration and Damage Assessment; K. McAbee, US Fish and Wildlife Service; C. Walsh, The University of Melbourne

Three case studies illustrate ways in which adaptive management has been used in ecological restorations that involve contaminants. The incorporation of risk assessment into adaptive management was critical. Contaminants addressed in these case studies include mercury (Hg), selenium (Se) and the suite of contaminants and physical disturbances delivered to streams by urban storm water runoff . All three cases emphasize the importance of broad stakeholder input early and consistently throughout decision analysis for adaptive management. Risk of contaminant exposure provided input to the decision analyses (e.g. selenium exposure to endangered razorback suckers, Stewart Lake; multiple contaminants in urban stormwater runoff, Melbourne) and was balanced with the protection of resources deemed critical for a desired future state (e.g. preservation of old growth trees, South River). Monitoring efforts also played a critical role in the ability to conduct the decision analyses necessary for the adaptive management plans. For example, newer technologies applied in the Melbourne case provided a testable situation where contaminant concentrations and flow disturbance were reduced to support a return to good ecological condition. In at least one case (Stewart Lake), long term monitoring data are being used to document the potential effects of climate change on a restoration trajectory. All three cases used adaptive management for restoration efforts. Decision analysis formalized the process by which stakeholders arrived at the priorities for the sites, which together constituted the desired future condition towards which each restoration is aimed. Alternative models were developed that described in mechanistic terms how restoration can influence the system towards the desired future condition. Including known and anticipated effects of future climate scenarios in these models will make them robust to the long-term exposure and effects characteristics of contaminants in restored ecosystems.

What Made You Become a Successful Science Communicator? Tell us!

PC017 USEPA Safer Choice Program: How Labels Can Communicate Hazard-based Toxicity Data

S. Au, USEPA / Safer Choice Program

For products such as cleaners, consumers typically spend no more than a few seconds making purchasing decisions. Well-designed labels can be a useful way to quickly convey complex scientific messages to consumers. There is limited consumer understanding of the chemicals used in common household products. Product manufacturers are not always able to disclose sensitive proprietary information, and it is difficult to effectively translate scientific terminology for consumers. The USEPA Safer Choice Program assesses chemicals to ensure they meet stringent human and environmental health criteria and fulfill performance standards across various consumer product categories such as cleaners and detergents. Products that meet this standard qualify for an easily identifiable label that serves as a convenient way to inform consumers about the review and safety of all chemicals. The USEPA Safer Choice Program also maintains the Safer Chemical Ingredients List, a publically-available platform that can help product manufacturers find safer chemical alternatives that meet the criteria of the Safer Choice program. Thus far, more than 500 product manufacturers have collaborated with Safer Choice to evaluate products to carry the Safer Choice Label. The label can be found on around 2,000 household and industrial products. Building credible relationships with non-scientists across product sectors can be challenging. But the goal becomes attainable through collaboration that balances the interests of the broad range of the program's stakeholders. The USEPA Safer Choice label encourages the use of safer chemistry, while condensing complex toxicological data into a label that communicates with consumers. This label continues to use the latest scientific data and computer modeling programs to ensure the product works and uses the safest possible chemicals. As a result, the program builds trust in consumers that greener and safer products are just as effective as traditional products and provide additional consumer value.

PC018 Bitesize Science: Blogging with Oceanbites and Envirobites brings scientific research to broad audiences

C.A. McDonough, University of Rhode Island / Graduate School of Oceanography; D.E. Eberle, Geosyntec Consultants; L. Schifman, USEPA

Oceanbites.org and envirobites.org are science blogs where graduate students, postdoctoral researchers, and science professionals from around the world are come together to write compelling, jargon-free summaries of recent research papers to make them accessible to non-experts. The main objectives of these online communities are to, firstly, make new research more accessible to the non-expert public, and secondly, to provide scientists with the opportunity to practice honing messages for broad audiences. Journal articles are often extremely detailed and make use of jargon that can be difficult even for other scientists to parse, making them unapproachable for curious members of the public, and coverage of recent scientific publications by major news outlets is limited and often focuses on results, with little explanation of scientific methods. For this reason, the bites sites, which include not only oceanbites (oceanography) and envirobites (environmental science), but also astrobites (astrophysics) and chembites (chemistry), seek to distill the main messages from journal articles so that anyone with a high school-level science education can understand them. The sites have gained attention from educators, writers, and K-12 students as a resource for them to stay up-to-date with the latest findings in their fields of interest. Oceanbites, which was founded in 2013, currently has more than 20 graduate student authors from universities around the world contributing regularly to the site, and has a dedicated following on social media, with more than 10,000 Twitter followers, and more than 700 Facebook followers. The blog publishes one article per weekday as well as occasional posts on science communication and popular media. Other features to engage readers include biweekly Spanish translations and theme weeks focused on topics voted for by our readers. Envirobites is a new venture seeking to create accessible science content for all fields of environmental science and is currently seeking interested scientists who would like to develop their non-technical writing skills. Here, our journey in developing oceanbites and envirobites will be described, as will our work developing workshops in blog writing for undergraduate researchers, and our plans to involve our readers in ocean and environment-related citizen science projects.

PC019 Expert Science Communication at American Chemical Society

H. Plugge, 3E Company

The American Chemical Society (ACS) Experts program has helped me become a better science communicator. The program involves a wide range of experts selected by ACS based on their scientific training and/or specialty. ACS Experts are recruited from academia, government and industry. Identification of potential Experts is performed through observation of speakers at scientific congresses and/or scientists' previous presentation(s) for ACS, among others factors. I became an ACS Expert in the fall of 2016. ACS Experts undergo initial training aimed at improving their public speaking and communication skills. Mock media interviews are another training mechanism employed in the program, as are occasional improv sessions. Follow-up training is also provided, generally in conjunction with ACS national meetings. ACS Experts are used to provide input and/or commentary on scientific questions submitted to ACS from the media. Questions are generally posed by news outlets, including print, broadcast and online outlets. Generally these questions are posted on a deadline – a 2 hour turnaround is not uncommon! The ACS Communications office acts as the facilitator connecting media with ACS Experts, but the Experts' represent their own opinions in any interview (they do not represent or speak on behalf of ACS). As a hazard/risk assessment/regulatory toxicology expert, questions are all over the map; as you become familiar with your role as Expert your communication skills increase as does the ease with which you can span across various aspects of given topics. Questions have ranged from exposure levels at and effects from Superfund sites to acting as a scientific reviewer of an animated feature on the LD50 of Halloween candy. Another mechanism where ACS facilitates communication skills is through its weekly "Ask Me Anything" (AMA) Reddit sessions, www.reddit.com/r/science. In this online forum, any Reddit user can ask a question of the host, who addresses them during a pre-scheduled hour. Volunteer moderators ensure that questions and responses stay on topic. My AMA session on Regulatory Toxicology, proved popular: over 5,000 reactions/votes on about 500 questions within 2 hours of my starting the session. I did not get to all of the questions, but it was exciting to see how much an online audience takes interest in such technical and societally important topics.

PC020 SETAC: Science for Everyone Takes Activities and Comedy

W.J. Berry, K. Mulvaney, USEPA / Atlantic Ecology Division ORD NHEERL

We have all been there: the presenter is droning on, putting up facts and figures and equations, and speaking in an almost inhuman monotone. To survive, you read your email under your desk and send "sleepy" emojis to the person next to you. We have all also been to talks where the speaker is presenting similar material but where you have been engaged, attentive, and aching to hear more. In science communication, style matters. Without a clear message made relevant to the audience, with readable slides, and an engaging speaking voice, even the most compelling scientific findings may be boring and poorly received. But what to do if you are not Neil DeGrasse Tyson, with the *Cosmos* behind you? Include a little humor and novelty. For example, engage your audience through something unexpected like the "History of Sediment Toxicity Assessment as told Through Bumper Stickers". Also, a few light jokes or self-deprecating cracks help to lighten the mood and engage the audience. If you really need to get the audience into it, games work great for connecting people and science. Games can be used as teaching tools for everyone from third graders to partner organizations to fellow scientists. They can be as simple as a cooler of animals and a score sheet, or as complex as a computer simulator with a fancy model behind it. At the Atlantic Ecology Division, we have piloted a number of different games to engage the local community in our science. These include watershed models, sustainability challenges, the "Compost Challenge", and the "Sediment Game". We will share some of the most successful games and their communication success stories that can be adapted for use in your own communities or work. Keep in mind, like every tool, humor and games are not appropriate for every situation. Both need to be framed appropriately for your audience. Additionally, humor does not come naturally to everyone, and it can be overdone. Games can also be a lot of work. But, both humor and games can be great fun, and can help you communicate your science better. Check 'em out.

PC021 What we can learn from teaching risk communication to golf course superintendents

S.Z. Cohen, Environmental & Turf Services, Inc.

Federal pesticide registration is a comprehensive, science-driven process that can involve some cutting edge approaches to risk assessment. Golf course permitting decisions are usually made at the local and county levels, with only occasional involvement from state government, and rare involvement from the federal government on wetlands issues only. Negative public perception of pesticide risks usually permeates the public hearing process, despite the application of site-specific pesticide risk assessments. The US EPA's pesticide program does not take a strong leadership position communicating the science of pesticide exposure, toxicity, and risks. That leaves a vacuum that is often filled with non-peer-reviewed information obtained from the internet by pesticide skeptics. The purpose of this talk will be to share lessons that I have learned testifying on pesticide environmental science issues well over 70 times in a 40 year career, including teaching risk communication to golf course superintendents. Examples of actual public statements that I have had to rebut will be presented, along with the scientific rebuttal. The following topics may be discussed: the importance of exposure in risk assessment, and its distinction from hazard assessment; results of epidemiology studies, and the need to consider 'the whole everything'; the thoroughness of pesticide testing required by 40 CFR Part 158; endocrine system activity vs. endocrine disruption; the NOEL/NOEC concept; environmental fate modeling; etc. Finally, some advice will be given on how to deliver the message in public meetings and in news media interviews.

PC022 Thinking beyond the science: Teaching tools to help students understand and communicate the reciprocal relationship between science and policy

P.K. Sibley, University of Guelph / School of Environmental Sciences

In many university environmental science-based programs, students often graduate with little understanding about the important role of science in the development of environmental policy. Post-graduation, many students may therefore find themselves thrust into the world of environmental policy development with little understanding of how science can/should be applied and how to communicate new policies to the public. In an era where science seems to be increasingly under siege, the importance of drawing clear connections between science/scientific research and policy making, and the capacity to effectively communicate this relationship, seems more critical than ever. In this presentation, I will draw upon two effective teaching tools, scenario analysis and policy briefs, that I have incorporated into graduate level teaching to understand and communicate the important reciprocal relationship between science and policy. Scenario analysis is a process used to analyze possible future events based on current and historical scientific understanding by considering alternative possible outcomes, deciding which options are desired and/or achievable, and then developing commensurate policy options that will achieve those outcomes. Policy briefs are typically short (1-4 page) documents that may be neutral in approach (articulate the state of science and the need for new policy or changes to existing policies in light of emerging knowledge) or advocate a particular policy position that is of interest to its creator(s). I will also consider the potential application of these communication tools as an effective approach to make SETAC science more accessible to its members, to decision-makers, and to society.

PC023 Presence of Antibiotics in Urban/Rural Streams of North Carolina: How public outreach can amend its prevalence

A.D. Gray, A.E. Hershey, University of North Carolina at Greensboro / Biology

The United State Environmental Protection Agency or US EPA has classified pharmaceuticals and personal care products (PPCPs) as contaminants or emerging concern. These compounds have shown over time to be detected continuously at low levels in surface waters and may serve as potential hazards for life in aquatic systems. Antibiotics make up the largest class of pharmaceuticals, with their use being applicable to human and veterinarian purposes. The presence of antibiotics in these systems plays a critical role in antibiotic resistance spreading amongst a wide spread of bacteria. Antibiotic resistance occurs naturally, but misuse of antibiotics in humans and animals is accelerating the process. Infections such as, pneumonia, tuberculosis, blood poisoning and gonorrhea are becoming harder to treat due to this antibiotic resistance. Much of the pathways that lead to antibiotics entering the environment stem from human misuse/mishandling. This misuse/mishandling stems from improper disposal of antibiotics, prescribing antibiotics to patients who do not need antibiotics, sharing antibiotics, runoff, and from waste water treatment facilities. Information concerning the proper disposal of antibiotics along with the implication of antibiotic contamination into the environment is not known by most of the public. These effects can be mediated if more of the public is aware and educated about how to reduce the amount of antibiotics released into the environment. Current work supported by UNC Greensboro and NC Sea Grant/WRRI has detected several human and veterinarian antibiotics in Guilford County, NC. Supported research is also investigating the presence of antibiotics in urban and rural systems in Guilford, Randolph and Alamance Counties, NC. Results from this work will be useful in engaging residents at town meetings, high school student outreach, reinforcing policies regarding emerging contaminants, and encouraging the appropriate use and disposal of quality medicines, and generally making more information available to citizens outside the science community. Hopefully, these efforts will aid in bridging the gap between the scientist, physicians, and the general public.

PC024 Science communication: Thinking globally and acting locally

L. Paulik, Oregon State University / Environmental and Molecular Toxicology

Effective science communication is becoming increasingly important in the modern age of technology and politicized science. Scientists are trained how to talk about science with other scientists, but are often left to their own devices to learn how to talk about science with anyone else. This disparity creates a troubling communication gap between scientists and non-scientists. When scientists can't, or don't, communicate about their work and why it's important, their work is taken less seriously by the public. When public opinion doesn't prioritize science, there is less financial support for scientific research to continue, and scientific progress is hindered. Limiting the audience that learns about research advances also limits the impact those findings can have. During graduate school I participated in community-engaged research projects. In these I was able to talk directly with community members about the research projects I was working on, and about how the results of these projects might impact their lives. Through these interactions I also learned some of the reasons that scientists don't always communicate with the public about their work: it's really hard, there is minimal guidance, and often there is little outreach-specific financial support. I found the challenge of effectively communicating with community members about our research fascinating, so I began to seek every opportunity I could to gain more experience communicating science to non-scientists. I applied for a Science Communication Fellowship with my local science museum and volunteered to lead K-12 science outreach at my university. Within SETAC I worked to increase SETAC's public science outreach and to increase chances for students to learn how to effectively communicate science to diverse audiences, and I got involved in the SCIRIC. More recently I have found volunteering locally to be the best way to have meaningful conversations with non-scientists. For the past few months I've volunteered with a local science museum, a local community college, and a non-profit called Girls Inc. These venues have set the stage for candid conversations about major issues in environmental science. I believe that if more scientists prioritized seeking connections like these at the local level, this would increase the public's trust of scientists. This local effort would encourage society to put more resources into science, on national and global scales.

H

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J

K

L

M

Q

R

T

AFFILIATION INDEX

SETAC North America 38th Annual Meeting | 409

V

W

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