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EMS Abstracts

Supplement to *Environmental and Molecular Mutagenesis*

In this issue: Abstracts from the Environmental Mutagen Society 42nd Annual Meeting, October 15–19, 2011, Montréal, Québec, Canada
Program Chair: Catherine B. Klein

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Environmental Mutagen Society 42nd Annual Meeting

Environmental Impacts on the Genome and Epigenome: Mechanisms and Risks

October 15–19, 2011

Hilton Montréal Bonaventure Montréal, Québec, Canada

Program Chair: Catherine B. Klein, Ph.D.

EMS Headquarters
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Annual Meeting Agenda

Friday, October 14

4:00 PM–6:00 PM — **Inscription**

REGISTRATION

Saturday, October 15

10:00 AM–12:30 PM — **Longueuil**

EMS EXECUTIVE BOARD MEETING

11:00 AM–6:00 PM — **Inscription**

REGISTRATION

1:00 PM–4:30 PM — **Fontaine C**

EMS COUNCIL MEETING

3:00 PM–6:00 PM — **Inscription 2**

SPEAKER READY ROOM OPEN

3:30 PM–5:00 PM — **Mont-Royal**

SATURDAY WORKSHOP

MENTORING: SKILLS DEVELOPMENT

Chairperson: ***Ofelia A. Olivero,*** *National Cancer Institute, NIH*

5:00 PM–5:30 PM — **Mont-Royal**

NIH FUNDING OPPORTUNITIES FORUM

Chairperson: ***Jeffrey L. Schwartz,*** *University of Washington*

5:00 PM — **Introduction**
Jeffrey L. Schwartz, *University of Washington*

5:05 PM — **Looking to the Future at the NIEHS**
Richard P. Woychik, *National Institute of Environmental Health Sciences*

5:30 PM–7:30 PM — **Le Portage**

WELCOME RECEPTION AND STUDENT AND NEW INVESTIGATOR POSTER SESSION

Supported in part by: Genetic Toxicology Association

Sunday, October 16

7:00 AM–5:00 PM — **Inscription**

REGISTRATION

7:00 AM–5:00 PM — **Inscription 2**

SPEAKER READY ROOM OPEN

7:00 AM–8:30 AM — **BREAKFAST MEETINGS**

BREAKFAST PICK-UP FOR MEETINGS — **Net Café**
(Ticket Required—Breakfast Available at 6:45 AM)

APPLIED GENETIC TOXICOLOGY SPECIAL INTEREST GROUP — **Fontaine F**

Leaders: ***Krista L. Dobo,*** *Pfizer Global R&D and* ***Patricia A. Escobar,*** *Boehringer Ingelheim Pharmaceuticals*

Underlined author indicates presenter.

7:00 AM — **Breakfast and Discussion on General Activities of the SIG**

7:30 AM — P71 — **Investigating the Utility of the Muta™Mouse Transgenic Rodent Assay for Regulatory Decision-Making: A Multi-Endpoint Comparison of Several *In Vivo* False Negatives**
Long AS[1,2], Lemieux CL[2], Dertinger S[3], White PA[1,2]. [1]Department of Biology, University of Ottawa, Ottawa, ON, Canada, [2]Mechanistic Studies Division, Environmental and Radiation Health Sciences Directorate, HECSB, Health Canada, Ottawa, ON, Canada, [3]Litron Laboratories, Rochester, NY, United States

8:00 AM — SG1 — **Further Characterization of the Genotoxic Effects of Phenolphthalein (PHT)**
Pamela L. Heard, *Pfizer Global Research and Development*

ENVIRONMENTAL GENETIC TOXICOLOGY SPECIAL INTEREST GROUP — **Fontaine G**

Leaders: ***Christopher M. Somers,*** *University of Regina and* ***Carol D. Swartz,*** Integrated Laboratory Systems

7:00 AM — **Welcome and Introduction**

7:15 AM — SG2 — **National Center for Radioecology (NCoRE): A New Network of Excellence for Environmental Radiation Risk Reduction and Remediation**
Stacey Lance, *Savannah River Ecology Lab*

7:30 AM — SG3 — **Nuclear Power Generation and Environmental Disasters: Lessons Learned from Chernobyl and Fukushima Daiichi**
Yuri E. Dubrova, *University of Leicester*

8:10 AM — **Group Discussion**

TRANSGENIC AND *IN VIVO* MUTAGENESIS SPECIAL INTEREST GROUP

Fontaine H

Leaders: ***Mugimane G. Manjanatha*** *and* ***Nan Mei,*** *National Center for Toxicological Research, U.S. FDA*

Presenting author is underlined.

7:00 AM		**General Business and Election of a New Chair and Co-Chair**
7:15 AM	P115	**A Population-Level Genetic Model of Low Dose Co-Exposures Reveals a Genetic Basis for Increased Cancer Susceptibility** *DeSimone MC, Mashburn Z, Wadsworth K, Patisaul H, Threadgill DW. North Carolina State University, Raleigh, NC, United States*
7:30 AM	19	**Role of the Circadian Clock in UV-Induced Skin Carcinogenesis** *Gaddameedhi S, Selby CP, Sancar A. University of North Carolina, Chapel Hill, NC, United States*
7:45 AM	P73	**Simultaneous Measurements of DNA Adducts, Pig-a and lacZ Mutations, and Micronuclei Induced by 3 PAHs** *Lemieux CL[1], Arlt VM[2], Dertinger SD[3], Phillips DH[2], White PA[1]. [1]Mechanistic Studies Division, Environmental Health Sciences and Research Bureau, Health Canada, Ottawa, ON, Canada, [2]Section of Molecular Carcinogenesis, Institute of Cancer Research, Sutton, Surrey, United Kingdom, [3]Litron Laboratories, Rochester, NY, United States*
8:00 AM	P74	***In Vivo*** **Multi-Endpoint Investigation of Chlorambucil Genotoxicity: Pig-a Mutation, Micronucleus, and Comet Assays** *Torous D[1], Vasquez M[2], Phonethepswath S[1], Weller P[1], Avlasevich S[1], Mareness J[1], Bemis J[1], Sivers C[2], Dewhurst N[2], MacGregor J[3], Dertinger S[1]. [1]Litron Laboratories, Rochester, NY, United States, [2]Helix3, Morrisville, NC, United States, [3]Toxicology Consulting Services, Arnold, MD, United States*
8:15 AM	P126	**Genotoxicity of Styrene-Acrylonitrile Trimer in Brain, Liver, and Blood Cells of Weanling F344 Rats** *Hobbs CA[1], Chhabra RS[3], Recio L[1], Winters J[1], Shepard K[1], Allen P[1], Streicker M[2], Witt KL[3]. [1]Genetic and Molecular Toxicology Division, ILS, Inc., Research Triangle Park, NC, United States, [2]Investigative Toxicology Division, ILS, Inc., Research Triangle Park, NC, United States, [3]National Toxicology Program, NIEHS, Research Triangle Park, NC, United States*

8:30 AM–9:30 AM **Outremont**

PLENARY LECTURE 1 (PL1)

THE CANCER EPIGENOME

Chairperson: ***Janet E. Baulch,*** *University of Maryland, Baltimore*

Lecturer: ***Peter A. Jones,*** *Norris Comprehensive Cancer Center, USC*

9:45 AM–12:15 PM **Mont-Royal**

SYMPOSIUM 1

EPIGENETICS: DNA MODIFICATIONS AND REPAIR

Chairpersons: ***Alfonso Bellacosa,*** *Fox Chase Cancer Center and* ***Jacquetta M. Trasler,*** *McGill University*

Organized by the DNA Repair and Mutagenic Mechanisms Special Interest Group and the Epigenetics Special Interest Group

Underlined author indicates presenter.

9:45 AM	S1	**DNA Methylation in Mammalian Development** ***Jacquetta M. Trasler,*** *McGill University*
10:15 AM	S2	**Role of 5-hydroxymethylcytosine in Reprogramming the Paternal Genome in the Zygote** ***Piroska E. Szabó,*** *Beckman Research Institute, City of Hope*
10:45 AM		**Break**
11:00 AM	S3	**Active DNA Demethylation by Thymine DNA Glycosylases** ***Alfonso Bellacosa,*** *Fox Chase Cancer Center*
11:30 AM	S4	**Prenatal Exposures to Environmental Pollutants and Epigenetics in Children** *Holland N, Yosefi P, Aguilar R, Quach H, Huen K, Bradman A, Venkat S, Barcellos L, Eskenazi B. University of California, Berkeley, Berkeley, CA, United States*
11:45 AM	S5	**Genomic and Epigenomic Alterations in Cells Exhibiting Radiation-Induced Genomic Instability** *Ding D, Aypar U, Tiper I, Goetz W, Baulch JE. University of Maryland, Baltimore, MD, United States*
12:00 NOON		**Discussion**

9:45 AM–12:15 PM **Outremont**

SYMPOSIUM 2

APPLICATION OF TOXICOGENOMICS IN REGULATORY DECISION-MAKING

Chairpersons: ***Guosheng Chen*** *and* ***Kathy Hughes,*** *Health Canada*

Organized by Health Canada

9:45 AM	S6	**Challenges to the Use of Emerging Technologies in Regulatory Risk Assessment** ***Kathy Hughes,*** *Health Canada*
9:55 AM	S7	**Application of Toxicogenomics in Health Risk Assessment** ***Michael D. Waters,*** *Integrated Laboratory Systems, Inc.*
10:20 AM	S8	**Comparison of Transcriptional Benchmark Doses to Traditional Cancer and Non-Cancer Endpoints for Quantitative Benzo(a)pyrene** ***Carole L. Yauk,*** *Health Canada Genomics Working Group*
10:40 AM	S9	**Opportunities for Integrating Toxicogenomic Approaches for Regulatory Decision-Making** ***Elaine M. Faustman,*** *University of Washington*
11:00 AM		**Break**

11:15 AM S10 **An Approach to Using Toxicogenomic Data in Risk Assessment: Dibutyl Phthalate Case Study**
Susan Y. Euling, *U.S. Environmental Protection Agency*

11:35 AM S11 **Integration of Genomic Biomarkers in Cancer Risk Assessment Paradigm**
Jiri Aubrecht, *Pfizer Inc.*

11:55 AM S12 **Practical Considerations for the Application of Toxicogenomics to Risk Assessment: Early Experience, Current Drivers, and a Path Forward**
Darrell R. Boverhof, *The Dow Chemical Company*

12:00 NOON–4:00 PM **Fontaine B**

ACCESS TO EXHIBIT HALL TO AFFIX POSTERS

12:15 PM–2:00 PM

LUNCH ON YOUR OWN

12:30 PM–1:45 PM **Fontaine F**

STUDENT AND NEW INVESTIGATOR BRUNCH

(Advance Registration Required)

2:00 PM–4:30 PM **Mont-Royal**

SYMPOSIUM 3

ETIOLOGY OF AUTISM: GENETICS, EPIGENETICS, AND THE ENVIRONMENT

Chairpersons: ***Janet E. Baulch,*** *University of Maryland, Baltimore and* ***Barbara S. Shane,*** *Barbara Shane Consulting*

Organized by the Epigenetics Special Interest Group

Contributing Sponsor: March of Dimes Birth Defects Foundation

Supported in part by: Autism Speaks

2:00 PM S13 **Overview of the Etiology of Autism**
Alycia Halladay, *Autism Speaks*

2:30 PM S14 **Etiology of Autism: A Role for Epigenetics?**
Rosanna Weksburg, *University of Toronto*

3:00 PM **Break**

3:15 PM S15 **Genomic Rearrangements in Autism: The Contribution of Copy Number Loss and Gain to the Etiology of Autism Spectrum Disorders**
Simon G. Gregory, *Duke University*

3:45 PM S16 **The Epigenetic Interface of Genetic and Environmental Risk Factors in the Etiology of Autism**
Janine LaSalle, *University of California, Davis School of Medicine*

4:15 PM **Discussion**

2:00 PM–4:30 PM **Outremont**

SYMPOSIUM 4

GENOTOXIC IMPURITIES: THE CURRENT ENVIRONMENT AND FUTURE POSSIBILITIES

Chairpersons: ***Michelle Kenyon,*** *Pfizer Inc. and* ***Laura L. Custer,*** *Bristol-Myers Squibb*

Organized by the Applied Genetic Toxicology Special Interest Group

2:00 PM S17 **Mutagenic Impurities: Introduction to the Current Environment and On-Going Initiatives**
David De Antonis, *Pfizer Inc.*

2:30 PM S18 **Case Studies Illustrating Validation of *In Silico* Prediction of Chemicals for Mutagenicity and Calculation of Compound-Specific Threshold of Toxicological Concern (TTC)**
Vijay Reddy, *Merck Research Laboratories*

3:00 PM **Break**

3:15 PM S19 **Dose-Response Relations: The Impact of Non-Linearity**
Melanie Guerard, *F. Hoffmann-La Roche Ltd.*

3:45 PM S20 **Application of the *In Vivo* Pig-a Mutagenicity Assay for Assessing Dose-Response Relationships**
Stephen D. Dertinger, *Litron Laboratories*

4:15 PM **Discussion**

4:30 PM–6:30 PM **Fontaine B**

POSTER SESSION 1 AND EXHIBITS

Odd numbered abstracts attended.

6:30 PM **COMMITTEE MEETINGS**

2012 PROGRAM COMMITTEE (FIRST MEETING) **Longueuil**

MEMBERSHIP AND PROFESSIONAL DEVELOPMENT COMMITTEE **Pointe Aux Trembles**

PUBLIC RELATIONS AND COMMUNICATIONS COMMITTEE **Jacques Cartier**

PUBLICATION POLICY COMMITTEE **Fundy**

7:30 PM **COMMITTEE MEETING**

SPECIAL INTEREST GROUP LEADERS **Pointe Aux Trembles**

Monday, October 17

7:00 AM–5:00 PM **Inscription**

REGISTRATION

7:00 AM–5:00 PM **Inscription 2**

SPEAKER READY ROOM OPEN

7:00 AM–8:30 AM **BREAKFAST MEETINGS**

BREAKFAST PICK-UP FOR MEETINGS **Net Café**
(Ticket Required—Breakfast Available at 6:45 AM)

DNA REPAIR AND MUTAGENIC MECHANISMS SPECIAL INTEREST GROUP **Fontaine F**
Leaders: ***Mats Ljungman,*** *The University of Michigan Medical School and* ***Joann B. Sweasy,*** *Yale University School of Medicine*

7:00 AM		**Discuss DNA Repair Topics for the 2012 Meeting**
7:40 AM		**Hot Topic: DNA Repair and the Cancer Genome Atlas**

HERITABLE MUTATION AND DISEASE SPECIAL INTEREST GROUP **Fontaine G**
Leaders: ***Francesco Marchetti*** *and* ***Carole Yauk,*** *Health Canada*

Underlined author indicates presenter.

7:00 AM		**General Business and Election of a New Co-Chair**
7:15 AM	P44	**The Effect of Advanced Paternal Age on Genetic Risks Is Mediated through Dysregulation of HRAS Signalling in the Testis** *Goriely A, McGowan SJ, Pfeifer S, Itani A, McVean GAT, Wilkie AOM. University of Oxford, Oxford, United Kingdom*
7:30 AM		**Committee on Germ Cell Mutagens** ***David DeMarini,*** *U.S. Environmental Protection Agency*
8:00 AM		**Roundtable Discussion**

RISK ASSESSMENT SPECIAL INTEREST GROUP **Fontaine H**
Leaders: ***David A. Eastmond,*** *University of California, Riverside and* ***Nagu Keshava,*** *U.S. Environmental Protection Agency*

7:00 AM		**Welcome and Announcements**
7:10 AM	SG4	**The U.S. Tox21 Effort: Current Status** ***Kristine L. Witt,*** *National Institute of Environmental Health Sciences*
7:30 AM	SG5	**Transcript Profiling to the Animal Cancer Phenotype: Maintaining the Gold Standard but Shortening the Cancer Bioassay** ***Michael D. Waters,*** *Integrated Laboratory Systems, Inc.*
7:50 AM	SG6	**Current and Future Direction of Integrated Risk Information Systems Program** ***Vincent J. Cogliano,*** *U.S. Environmental Protection Agency*
8:10 AM		**Business and Discussion**

8:30 AM–6:30 PM **Fontaine B**

POSTERS AND EXHIBITS OPEN

8:30 AM–9:30 AM **Outremont**

PLENARY LECTURE 2 (PL2)

DNA DAMAGE AND REPAIR IN CANCER: FROM BASIC SCIENCE TO ANTI-CANCER TREATMENTS

Chairperson: ***Mats Ljungman,*** *The University of Michigan Medical School*

Lecturer: ***Thomas Helleday,*** *Gray Institute for Radiation Oncology and Biology*

9:45 AM–12:15 PM **Mont-Royal**

SYMPOSIUM 5

MECHANISMS AND ROLES OF PARP IN RESPONSE TO ENVIRONMENTAL GENOTOXINS

Chairpersons: ***Robert W. Sobol, Jr.,*** *University of Pittsburgh Cancer Institute and* ***Guy G. Poirier,*** *Laval University*

Organized by the DNA Repair and Mutagenic Mechanisms Special Interest Group

Supported in part by: Abbott Laboratories, Global Pharmaceutical Research and Development and **Trevigen, Inc.**

9:45 AM	S21	**Programming of DNA Damage Signaling and Repair by Poly(ADP-ribose)** ***Guy Poirier,*** *Laval University*
10:05 AM	S22	**Targeted Therapy of Breast Cancer Using PARP Inhibitors** ***James M. Ford,*** *Stanford University*
10:25 AM	S23	**NAD$^+$-Dependent Gene Regulation by PARP-1** ***W. Lee Kraus,*** *University of Texas Southwestern Medical Center at Dallas*
10:45 AM		**Break**—*In the Exhibit Hall*
11:00 AM	S24	**Analysis of the NAD Metabolome in Response to DNA Alkylation** ***Charles Brenner,*** *University of Iowa*
11:20 AM	S25	**Role of PARP Inhibitors in ATM Deficient Human Malignancies** ***Susan Lees-Miller,*** *University of Calgary*
11:40 AM	S26	**Temporal and Spatial Resolution of PARP Activation-Induced Cellular Energy Modulation** ***Robert W. Sobol,*** *Jr., University of Pittsburgh Cancer Institute*
12:00 NOON		**Discussion**

9:45 AM–12:15 PM **Outremont**

SYMPOSIUM 6

ENVIRONMENTAL EXPOSURES' IMPACT ON MALE GERM CELLS AND CONSEQUENCES TO THE EMBRYO: IS THE PREDOMINANT DAMAGE GENETIC/EPIGENETIC?

Chairpersons: ***Francesco Marchetti,*** *Health Canada and* ***Bernard Robaire,*** *McGill University*

Organized by the Heritable Mutations Special Interest Group

9:45 AM	S27	**Impact of Paternal Drug Exposures on Progeny Outcome: The Roles of Genetic *versus* Epigenetic Mechanisms** ***Barbara F. Hales,*** *McGill University*
10:15 AM	S28	**Role of Histone Modifications in Male Germ Cell Development and Progeny Outcome** ***Sarah Kimmins,*** *McGill University*
10:45 AM		**Break**—*In the Exhibit Hall*
11:00 AM	S29	**Effects of Dietary Folic Acid Intake on Somatic and Germ Cells within and across Generations** ***Michael G. Wade,*** *Health Canada*
11:30 AM	S30	**Environmental Organochlorine Exposures and Sex Chromosome Disomy in Human Sperm** ***Melissa J. Perry,*** *The George Washington University*
12:00 NOON		**Discussion**

12:15 PM–2:00 PM

LUNCH ON YOUR OWN

12:15 PM–12:30 PM **Outremont Foyer**

BOXED LUNCH PICK-UP

(For EMS Business Meeting and Town Hall)

12:30 PM–1:45 PM **Outremont**

EMS BUSINESS MEETING AND TOWN HALL

(Boxed Lunches Available)

2:00 PM–4:30 PM **Mont-Royal**

SYMPOSIUM 7

APPLICATION OF EPIGENETICS AND EPIGENOMICS APPROACHES IN THE INVESTIGATION OF HUMAN DISEASE

Chairpersons: ***Jennifer C. Sasaki,*** *Alkermes, Inc. and* ***Sundaresan Venkatachalam,*** *National Institute of Dental and Craniofacial, NIH*

Organized by the New Technologies Special Interest Group

2:00 PM		**Introduction** ***Sundaresan Venkatachalam,*** *National Institute of Dental and Craniofacial, NIH*
2:15 PM	S31	**Coordination of Regulation of DNMT1 Stability** ***W. David Sedwick,*** *Case Western Reserve University*
2:45 PM	S32	**Translating Epigenetic Biology into Novel Treatments for Cancer** ***Owen A. O'Connor,*** *NYU Langone Medical Center*
3:15 PM		**Break**—*In the Exhibit Hall*
3:30 PM	S33	**Epigenetic Epidemiology: Examples from Alzheimer's Disease** ***Laura S. Rozek,*** *University of Michigan*
4:00 PM	S34	**Emerging Science and Technologies to Explore Epigenetic Mechanisms: Better Understanding of Human Diseases** ***Winnie Wan-yee Tang,*** *Johns Hopkins Bloomberg School of Public Health*

2:00 PM–4:30 PM **Outremont**

SYMPOSIUM 8

SITE OF CONTACT AND SYSTEMIC EFFECTS OF FORMALDEHYDE EXPOSURE

Chairpersons: ***Luoping Zhang,*** *University of California, Berkeley and* ***James Swenberg,*** *University of North Carolina*

Contributing Sponsor: National Institute of Environmental Health Sciences

2:00 PM		**Introduction** ***Luoping Zhang,*** *University of California, Berkeley*
2:05 PM	S35	**Evaluation of Formaldehyde As a Potential Cause of Human Leukemia** ***Bernard D. Goldstein,*** *University of Pittsburgh*
2:30 PM	S36	**Detection of Formaldehyde-Induced Bone Marrow Toxicity and Allergic Asthma in Experimental Animal Models** ***Xu Yang,*** *Central China (Huazhong) Normal University*
2:55 PM	S37	**Potential Genetic and Epigenetic Effects of Formaldehyde** ***James Swenberg,*** *University of North Carolina*
3:20 PM		**Break**—*In the Exhibit Hall*
3:35 PM	S38	**Reproductive and Developmental Toxicity of Formaldehyde: A Systematic Review** ***Luoping Zhang,*** *University of California, Berkeley*
4:00 PM		**Discussion**

2:00 PM–4:30 PM **Verdun**

PLATFORM SESSION 1

DNA REPAIR

Chairpersons: ***Bevin P. Engelward,*** *Massachusetts Institute of Technology and* ***Kandace J. Williams,*** *University of Toledo College of Medicine*

Presenting author is underlined.

2:00 PM	1	**Oligonucleotide Retrieval: A Versatile Technology for Calibrating the Fidelity of DNA Synthetic Processes *In Vivo*** *Shen JC[1], Fox EJ[1], Loeb LA[1,2]. [1]Department of Pathology, University of Washington, Seattle, WA, United States, [2]Department of Biochemistry, University of Washington, Seattle, WA, United States*

2:15 PM 2 **Comet-Fish to Sensitively Assess Global and Transcription-Coupled Repair of DNA Lesions**
Spivak G[1], Guo J[1], Hanawalt PC[1]. [1]Stanford University, Stanford, CA, United States

2:30 PM 3 **Chemotherapeutic Alkylation Treatment: Tumor Cell Response**
Kim S[1], Williams KJ[1]. [1]University of Toledo College of Medicine, Toledo, OH, United States

2:45 PM 4 **Alkylation Sensitivity Screens Reveal a Cross-Species Functionome**
Svilar D[1], Brown A[1], Tang JB[1], McDonald PR[1], Shun TY[1], Wang XH[1], Lazo JS[1], Begley TJ[2], Sobol RW[1]. [1]University of Pittsburgh, Pittsburgh, PA, United States, [2]University at Albany, State University of New York, Rensselaer, NY, United States

3:00 PM 5 **Structure and Substrate Specificity of a Mouse NEIL3 Ortholog**
Liu M[1], Imamura K[1], Averill A[1], Jaruga P[2], Zhao X[3], Burrows CJ[3], Dizdaroglu M[2], Doublié S[1], Wallace SS[1]. [1]Department of Microbiology and Molecular Genetics, The Markey Center for Molecular Genetics, University of Vermont, Burlington, VT, United States, [2]Chemical Science and Technology Laboratory, National Institute of Standards and Technology, Gaithersburg, MD, United States, [3]Department of Chemistry, University of Utah, Salt Lake City, UT, United States

3:15 PM **Break**—*In the Exhibit Hall*

3:30 PM 6 **Sumoylation of Ntg1 and the Regulation of Base Excision Repair in *Saccharomyces cerevisiae***
Swartzlander DB[1], Powers HR[1], Corbett AH[1], Doetsch PW[1,2]. [1]Emory University, Department of Biochemistry, Atlanta, GA, United States, [2]Winship Cancer Institute, Atlanta, GA, United States

3:45 PM 7 **Radiation Mitigators Yel1 and Yel2 Inhibit Radiation-Induced Error-Prone Microhomology-Mediated Recombination**
Scuric Z[1], Farabi N[1], Schiestl RH[1]. [1]David Geffen School of Medicine at UCLA, Los Angeles, CA, United States

4:00 PM 8 **FANCJ Functions in Error-Free UV DNA Repair**
Guillemette SS[1], Peng M[1], Cantor SB[1]. [1]University of Massachusetts Medical School, Worcester, MA, United States

4:15 PM 9 **Unified Model for Fanconi Anemia and Breast Cancer Gene-Network in Protecting Stalled DNA Replication Forks**
Schlacher K[1,2], Jasin M[1]. [1]Memorial Sloan-Kettering Cancer Center, New York, NY, United States, [2]University of California, Los Angeles, Los Angeles, CA, United States

4:30 PM–6:30 PM **Fontaine B**

POSTER SESSION 2 AND EXHIBITS

Even numbered abstracts attended.

6:30 PM **COMMITTEE MEETINGS**

ALEXANDER HOLLAENDER OUTREACH COMMITTEE **Fundy**

AWARDS AND HONORS COMMITTEE **Longueuil**

EDUCATION, STUDENT AND NEW INVESTIGATOR AFFAIRS COMMITTEE **Pointe Aux Trembles**

FINANCE AND RESOURCE COMMITTEE **Jacques Cartier**

Tuesday, October 18

7:00 AM–5:00 PM **Inscription**

REGISTRATION

7:00 AM–5:00 PM **Inscription 2**

SPEAKER READY ROOM OPEN

7:00 AM–8:30 AM **BREAKFAST MEETINGS**

BREAKFAST PICK-UP FOR MEETINGS **Net Café**
(Ticket Required—Breakfast Available at 6:45 AM)

EXECUTIVE BOARD MEETING **Longueuil**

EPIGENETICS SPECIAL INTEREST GROUP **Fontaine F**
Leaders: ***Janet E. Baulch,*** *University of Maryland, Baltimore and* ***Dana C. Dolinoy,*** *University of Michigan*

Underlined author indicates presenter.

7:00 AM **SIG Business**

7:20 AM P49 **Timing of Early Developmental Cigarette Smoke Exposure Determines Epigenetic and Gene Expression Changes**
Lyon JL, Gordon T. New York University School of Medicine, Tuxedo, NY, United States

7:40 AM P50 **Epigenetics of Parental Exposure to Environmental Mutagens**
Dubrova YE. Department of Genetics, University of Leicester, Leicester, United Kingdom

8:05 AM SG7 **Challenges of QAQC in the Analyses of Epigenetic Markers in Human Studies**
Nina Holland, *University of California, Berkeley*

NEW TECHNOLOGIES SPECIAL INTEREST GROUP **Fontaine G**
Leaders: ***Brinda Mahadevan,*** *Abbott Laboratories and* ***Jennifer C. Sasaki,*** *Alkermes, Inc.*

7:00 AM **Discussion on General Activities of the SIG**

7:20 AM SG8 **Cutting-Edge Sequence Discovery of Human Genetic Variation**
Richard A. Gibbs, *Baylor College of Medicine*

8:30 AM–9:30 AM **Outremont**

EMS AWARD LECTURE (PL3)

UNRAVELING THE MYSTERY OF A GLOBAL ENVIRONMENTAL DISEASE

Chairperson: ***Catherine B. Klein,*** *New York University School of Medicine*

Lecturer: ***Arthur P. Grollman,*** *Stony Brook University*

8:30 AM–12:00 NOON **Fontaine B**

POSTERS AND EXHIBITS OPEN

Last Chance to View Posters and Exhibits!

9:45 AM–12:15 PM **Mont-Royal**

SYMPOSIUM 9

WHOLE GENOME MUTATIONAL PROFILING OF ENVIRONMENTALLY-INDUCED CANCER

Chairpersons: ***James C. Fuscoe,*** *National Center for Toxicological Research, U.S. FDA and* ***David A. Wheeler,*** *Baylor College of Medicine*

Supported in part by: Global Occupational Toxicology, Abbott Quality and Regulatory

9:45 AM S39 **Updates on Next-Generation Sequencing and Bioinformatics**
David A. Wheeler, *Baylor College of Medicine*

10:10 AM S40 **Targeted Resequencing Using the PacBio RS Platform**
John D. McPherson, *Ontario Institute for Cancer Research*

10:35 AM S41 **Paired-End Sequencing Reveals Characteristic Patterns of Structural Variations in Epithelial Cancer Genomes**
Guillaume Bourque, *McGill University*

11:00 AM **Break**—*In the Exhibit Hall*

11:15 AM S42 **Landscape of Somatic Alterations in Cancer and Their Relationship to the Environment**
Kristian Cibulskis, *The Eli and Edythe L. Broad Institute*

11:40 AM S43 **The Human SNP Site-Frequency-Spectrum**
Richard A. Gibbs, *Baylor College of Medicine*

12:05 PM **Discussion**

9:45 AM–12:15 PM **Outremont**

SYMPOSIUM 10

TRANSCRIPTION AND GENETIC INSTABILITY

Chairpersons: ***Malcolm J. Lippert,*** *Saint Michael's College and* ***Mats Ljungman,*** *University of Michigan*

Organized by the DNA Repair and Mutagenic Mechanisms Special Interest Group

9:45 AM S44 **Role for Topoisomerase I in Transcription-Associated Mutagenesis**
Malcolm J. Lippert, *Saint Michael's College*

10:10 AM S45 **Transcription Impacts Genomic Stability *via* Multiple Mechanisms**
Nayun Kim, *Duke University Medical Center*

10:35 AM S46 **Transcription Associated Recombination at Replication Forks in Mammalian Cells**
Thomas Helleday, *Gray Institute for Radiation Oncology and Biology*

11:00 AM **Break**—*In the Exhibit Hall*

11:15 AM S47 **Transcription-Blockage by Lesions and Unusual DNA Structures**
Philip C. Hanawalt, *Stanford University*

11:45 AM S48 **The Transcriptome and the DNA Repairome**
Mats Ljungman, *University of Michigan*

9:45 AM–12:15 PM **Verdun**

SYMPOSIUM 11

LOW-DOSE MUTAGENESIS AND CARCINOGENESIS

Chairpersons: ***Errol Zeiger,*** *Errol Zeiger Consulting and* ***David M. DeMarini,*** *U.S. Environmental Protection Agency*

Supported in part by: ILSI-HESI IVGT Project Committee

9:45 AM S49 **Low-Dose Mutagenesis and Carcinogenesis: Why Is This Issue Important**
Errol Zeiger, *Errol Zeiger Consulting*

9:50 AM S50 **Low-Dose Mutagenicity Case Studies: MMS and MNU**
Lynn H. Pottenger, *The Dow Chemical Company*

10:15 AM S51 **Low-Dose *In Vivo* Study of Acrylamide**
Errol Zeiger, *Errol Zeiger Consulting*

10:40 AM S52 **Low-Dose Carcinogenicity Studies**
David M. DeMarini, *U.S. Environmental Protection Agency*

11:00 AM **Break**—*In the Exhibit Hall*

11:15 PM S53 **Analysis of Low-Dose Mutagenicity Responses and the Applicability of Genotoxicity Tests for Carcinogen Potency Predicition**
Lya Hernandez, *National Institute for Public Health and the Environment*

11:40 PM S54 **Dose-Response Issues in the Regulation of Chemicals**
Rita Schoeny, *U.S. Environmental Protection Agency*

12:05 PM **Discussion**

12:00 NOON–12:30 PM

POSTER REMOVAL

12:15 PM–2:00 PM

LUNCH ON YOUR OWN

12:30 PM–1:45 PM **LUNCH MEETING**

WOMEN IN THE EMS SPECIAL INTEREST GROUP **Fontaine F**

(Advance Registration Required)

Leaders: ***Janice M. Pluth,*** *Lawrence Berkeley National Laboratory and* ***Glenda J. Gentile,*** *University of Arizona*

12:30 PM **Networking**

12:45 PM **New Business**

12:55 PM SG9 **Women As Leaders in the Scientific Enterprise**
Geraldine L. Richmond, *COACh Program, University of Oregon*

1:30 PM **Networking**

2:00 PM–3:15 PM **Mont-Royal**

SYMPOSIUM 12

RISKS ASSOCIATED WITH INADVERTENT EXPOSURES TO PHARMACEUTICAL AND PRESCRIPTION DRUGS

Chairpersons: ***Brinda Mahadevan,*** *Abbott Laboratories and* ***Ainsley Weston,*** *Centers for Disease Control and Prevention*

Organized by the New Technologies Special Interest Group and the Applied Genetic Toxicology Special Interest Group

2:00 PM S55 **Inadvertent Exposures to Pharmaceutical Drugs: Overview**
Ainsley Weston, *National Institute for Occupational Safety and Health, CDC*

2:15 PM S56 **Risk-Based Manufacture of Pharmaceutical Products**
Brinda Mahadevan, *Abbott Laboratories*

2:35 PM S57 **Considerations for Risk Assessments of Genotoxic or Carcinogenic Impurities in Industry**
John Nicolette, *Abbott Laboratories*

2:55 PM S58 **Preventing Occupational Exposures to Antineoplastic Drugs in Health Care Settings**
Thomas H. Connor, *National Institute for Occupational Safety and Health, CDC*

2:00 PM–3:15 PM **Outremont**

SYMPOSIUM 13

USING DNA ADDUCTS IN RISK ASSESSMENT: APPROACHES, CONSIDERATIONS, AND SIGNIFICANCE

Chairpersons: ***Lynn H. Pottenger,*** *The Dow Chemical Company and* ***Annie Jarabek,*** *National Center for Environmental Assessment, U.S. EPA*

Primary Sponsor: ILSI-HESI DNA Adducts Project Committee

2:00 PM S59 **ILSI-HESI DNA Adducts Project Committee: Review of Case Study Outcomes: Tamoxifen, AFB_1, and VCI**
Lynn H. Pottenger, *The Dow Chemical Company*

2:25 PM S60 **Application of Decision Analytic Approach to Case Studies**
Annie Jarabek, *National Center for Environmental Assessment, U.S. EPA*

2:40 PM S61 **Mutagenesis and Repair of O^6- and N7-Alkylguanine Adducts**
Robert Fuchs, *Centre National de la Recherche Scientifique*

3:00 PM S62 **Relevance of DNA Adduct Approaches to Regulatory Risk Assessment**
Rita Schoeny, *U.S. Environmental Protection Agency*

2:00 PM–4:45 PM **Verdun**

PLATFORM SESSION 2

MUTAGENESIS AND CARCINOGENESIS MECHANISMS

Chairpersons: ***Patricia A. Escobar,*** *Boehringer Ingelheim Pharmaceuticals and* ***Ofelia A. Olivero,*** *National Cancer Institute, NIH*

Presenting author is underlined.

2:00 PM 10 **Subchronic Oral Exposure to Benzo[a]Pyrene Induces Changes in Gene Expression Associated with Cellular Transformation in Mouse Lungs: A Toxicogenomics Study**
Labib S[1], Williams A[2], Lemieux CL[2], White PA[2], Halappanavar S[2]. [1]University of Ottawa, Ottawa, ON, Canada, [2]Health Canada, Ottawa, ON, Canada

2:15 PM 11 **The Mouse Diversity Genotyping Array Profiles Tissue- and Genotype-Specific Mutations across the Mouse Genome**
Eitutis ST, Wishart AE, Hill KA. The University of Western Ontario, London, ON, Canada

2:30 PM 12 **DNA Structure-Induced Genetic Instability in Mammals**
Wang G, Vasquez K. University of Texas at Austin, Austin, TX, United States

2:45 PM 13 **Identification of Different Mechanisms of Non-Genotoxic Carcinogens Based on Gene Expression Profiling in Primary Mouse Hepatocytes**
Schaap MM[1,2], Jonker MJ[3], Zwart PE[1], Wackers P[3], van de Water B[4], Breit TM[3], Schoonen WG[5], Polman J[5], van Steeg H[1,2], Luijten M[1]. [1]National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands, [2]Department of Toxicogenetics, Leiden University Medical Center, Leiden, Netherlands, [3]MicroArray Department and Integrative Bioinformatics Unit, University of Amsterdam, Amsterdam, Netherlands, [4]Leiden Amsterdam Center for Drug Research (LACDR), Leiden University, Leiden, Netherlands, [5]Merck Sharp & Dome, Oss, Netherlands

3:00 PM 14 **Tissue Specificity of Aristolochic Acid-Induced Carcinogenesis Examined by ACB-PCR Quantification of H-*Ras* Codon 61 CTA Mutant Fraction**
Wang Y[1], McKim KL[1], Myers MB[1], Arlt VM[2], Parsons BL[1]. [1]National Center for Toxicological Research, U.S. FDA, Jefferson, AR, United States, [2]Institute of Cancer Research, Sutton, Surrey, United Kingdom

3:15 PM **Break**

3:30 PM 15 **Toxicogenomic Late Effects of Antineoplastic Therapies for Lymphomas**
Marcondes JPC[1], Torres BP[1], Niéro-Melo L[1], Gaiolla RD[1], Luisi FAV[2], Salvadori DMF[1]. [1]São Paulo State University, Botucatu, SP, Brazil, [2]São Paulo Federal University, São Paulo, SP, Brazil

3:45 PM 16 **Functional Studies of Single-Nucleotide Polymorphic Variants of Human Glutathione Transferase T1-1 Involving Residues in the Dimer Interface**
Josephy PD[1], Pan D[1], Ianni MD[1], Mannervik B[2,3]. [1]University of Guelph, Guelph, Canada, [2]Uppsala University, Uppsala, Sweden, [3]Stockholm University, Stockholm, Sweden

4:00 PM 17 **Analyzing the Relationship between Radiation-Induced Phospho-Protein Signaling and Surrogate Cancer Endpoints Using Novel Flow-Based Assays**
Whalen MK[1], Sridharan DM[1], Wilson W[1], Chapell L[2], Cucinotta FA[2], Pluth JM[1]. [1]Lawrence Berkeley National Laboratory, Berkeley, CA, United States, [2]Lyndon B. Johnson Space Center, Houston, TX, United States

4:15 PM 18 **Lethal Mutagenesis of HIV and Cancer Cells by Nucleoside Analogs**
Fox EJ[1], Shen JC[1], Prindle MJ[1], Harris KS[4], Mullins JI[3], Essigmann JM[5], Loeb LA[1,2]. [1]Department of Pathology, University of Washington, Seattle, WA, United States, [2]Department of Biochemistry, University of Washington, Seattle, WA, United States, [3]Department of Microbiology, University of Washington, Seattle, WA, United States, [4]Koronis Pharmaceuticals, Seattle, WA, United States, [5]Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA, United States

4:30 PM 19 **Role of the Circadian Clock in UV-Induced Skin Carcinogenesis**
Gaddameedhi S, Selby CP, Sancar A. University of North Carolina, Chapel Hill, NC, United States

3:15 PM–3:30 PM

BREAK

3:30 PM–4:45 PM **Mont-Royal**

SYMPOSIUM 14

ENVIRONMENTAL OIL CONTAMINATION: EFFECTS ON MARINE LIFE AND HUMAN LIFE

Chairpersons: ***Christopher M. Somers,*** *University of Regina and* ***Miriam C. Poirier,*** *National Cancer Institute, NIH*

Organized by the Molecular Epidemiology Special Interest Group and the Environmental Genetic Toxicology Special Interest Group

3:30 PM S63 **Voyage 2 of the Odyssey: The Impact of the Oil Crisis on Health Using Whales As an Indicator Species**
John Wise, *University of Southern Maine*

3:45 PM S64 **NIOSH Health Hazard Evaluation Conducted in the Aftermath of the Deepwater Horizon (DWH) Disaster**
Ainsley Weston, *National Institute for Occupational Safety and Health, CDC*

4:00 PM S65 **Are Seafood Safety Considerations Adequate to Prevent Chronic Health Effects of the Deep Water Horizon Oil Spill on Coastal Louisiana Residents?**
Patricia L. Williams, *University of New Orleans*

4:15 PM S66 **Overview of the Toxicity of the Oil Dispersant Corexit**
David M. DeMarini, *U.S. Environmental Protection Agency*

4:30 PM **Panel Discussion**

3:30 PM–4:45 PM **Outremont**

SYMPOSIUM 15

INTESTINAL MICROBIOTA: A KEY PLAYER IN OBESITY, GENOMIC INSTABILITY, AND LYMPHOMA

Chairperson: ***Robert H. Schiestl,*** *UCLA School of Medicine and Public Health*

3:30 PM S67 **Intestinal Microbiota Affect Genetic Instability, Longevity and Lymphoma Latency in Atm$^{-/-}$ Mice**
Robert H. Schiestl, *UCLA School of Medicine and Public Health*

4:00 PM S68 **The Intestinal Host-Microbial Ecosystem: A Systems Biology Approach to Inflammation and Cancer Risk**
Jonathan Braun, *David Geffen School of Medicine at UCLA*

4:15 PM S69 **Intestinal Microbiota Involved in Rheumatoid Arthritis**
Jose U. Scher, *New York University Langone Medical Center*

4:30 PM **Discussion**

5:30 PM–6:50 PM **Outremont**

PUBLIC LECTURE (L1)

CANCER IN BELUGA WHALES FROM THE ST. LAWRENCE ESTUARY, QUÉBEC, CANADA: A CASE OF "ONE HEALTH, ONE MEDICINE"

Lecturer: ***Daniel Martineau,*** *University of Montréal*

Wednesday, October 19

7:00 AM–1:00 PM **Inscription**

REGISTRATION

7:00 AM–9:30 AM **Inscription 2**

SPEAKER READY ROOM OPEN

7:00 AM–8:30 AM **BREAKFAST MEETINGS**

BREAKFAST PICK-UP FOR MEETINGS **Net Café**
(Ticket Required—Breakfast Available at 6:45 AM)

2012 PROGRAM COMMITTEE MEETING (SECOND MEETING) **Longueuil**

MOLECULAR EPIDEMIOLOGY SPECIAL INTEREST GROUP **Fontaine F**
Leaders: ***Miriam Poirier,*** *National Cancer Institute and* ***Radim Sram,*** *Institute of Experimental Medicine*

7:00 AM **Overview of Activities for the Past Year: Introduction of the Topics for the Current Meeting**
Miriam Poirier, *National Cancer Institute*

7:30 AM SG10 **An Overview of ChIP-chip Technology and Its Application in the Discovery of Thyroid Hormone Receptor Binding Sites in the Mouse Genome**
Martin Paquette, *Health Canada and Carleton University*

8:00 AM SG11 **MicroRNAs: Biology, Technology, and Toxicology**
Julie A. Bourdon, *Health Canada*

8:30 AM–9:15 AM **Outremont**

PLENARY LECTURE 4 (PL4)

MECHANISTIC BASIS OF RESISTANCE TO PCBs AND DIOXIN IN ATLANTIC TOMCOD FROM THE HUDSON RIVER

Chairperson: ***Christopher M. Somers,*** *University of Regina*

Lecturer: ***Isaac I. Wirgin,*** *New York University*

9:30 AM–12:15 PM **Mont-Royal**

SYMPOSIUM 16

THE MULTIPLICITY OF DNA POLYMERASES: A STRATEGY FOR MAINTAINING GENOME STABILITY

Chairpersons: ***Joann B. Sweasy,*** *Yale University School of Medicine and* ***Kristin A. Eckert,*** *Pennsylvania State University College of Medicine*

Organized by the DNA Repair and Mutagenic Mechanisms Special Interest Group

9:30 AM **Introduction**
Kristin A. Eckert, *Pennsylvania State University College of Medicine*

9:35 AM S70 **Novel Roles for Specialized DNA Polymerases in Repetitive DNA Replication**
Kristin A. Eckert, *Pennsylvania State University College of Medicine*

9:55 AM S71 **Regulation of DNA Polymerase Eta in Human Cells by Post-Translational Modifications**
Alan R. Lehmann, *University of Sussex*

10:20 AM S72 **Function and Control of Translesion DNA Polymerases**
Graham C. Walker, *Massachusetts Institute of Technology*

10:45 AM **Break**

11:00 AM S73 **DNA Polymerase Dysregulation in Cancer and Animal Models**
Richard D. Wood, *The University of Texas MD Anderson Cancer Center*

11:25 AM S74 **REV1 and DNA Polymerase ζ Maintain Genomic Stability by Promoting DNA Repair**
Christine E. Canman, *University of Michigan*

11:50 AM S75 **DNA Polymerases in Meiosis**
Joann B. Sweasy, *Yale University School of Medicine*

9:30 AM–12:45 PM **Verdun**

SYMPOSIUM 17

PERSPECTIVES ON THE EVALUATION AND INTERPRETATION OF MUTAGENICITY AND GENOTOXICITY DATA

Chairpersons: ***Babasaheb (Bob) R. Sonawane,*** *U.S. Environmental Protection Agency and* ***George R. Douglas,*** *Health Canada*

Contributing Sponsor: U.S. Environmental Protection Agency

9:30 AM S76 **Overview: Perspectives on the Evaluation and Interpretation of Mutagenicity and Genotoxicity Data**
Babasaheb (Bob) R. Sonawane, *U.S. Environmental Protection Agency*

9:45 AM S77 **Overview of IARC and IRIS Use of Genetic Toxicity and Mechanistic Data**
Vince Cogliano, *U.S. Environmental Protection Agency*

10:05 AM S78 **Dichloromethane: A Case Study for the Interpretation of Genetic Toxicity Data by IARC and the IRIS Program**
Catherine Gibbons, *U.S. Environmental Protection Agency*

10:25 AM S79 **Evaluation and Interpretation of Chromosome Aberration Data on Chromium Picolinate: A Dietary Supplement Ingredient**
Ramadevi Gudi, *U.S. Food and Drug Administration*

10:45 AM **Break**

11:00 AM S80 **Assessment of Mutagenicity and Carcingenicity: An Overview of Recent Health Canada Research in Support of Hazard and Risk Assessment**
Paul A. White, *Health Canada*

11:20 AM S81 **European Union Requirements and Their Consequences for Consumer Product Genotoxicity Safety Testing**
Stefan J. Pfuhler, *The Procter & Gamble Company*

11:40 AM S82 **Evaluation and Interpretation of Genotoxicity Data: An Industry Perspective**
B. Bhaskar Gollapudi, *The Dow Chemical Company*

12:00 NOON S83 **The Use of Mutagenicity Determinations in Risk Assessment: An Academic Perspective**
David A. Eastmond, *University of California, Riverside*

12:20 PM **Panel Discussion**
Michael D. Waters, *Integrated Laboratory Systems, Inc.*

12:45 PM–6:30 PM

FREE AFTERNOON

2:00 PM–5:00 PM **Fontaine C**

EMS COUNCIL MEETING

6:30 PM–10:30 PM **Outremont**

EMS BANQUET

Awards Presentation by Jeffrey L. Schwartz

Alexander Hollaender Award
Student Education Award
Student and New Investigator Travel Awards

Dancing

ENVIRONMENTAL MUTAGEN SOCIETY 42ND ANNUAL MEETING ABSTRACTS

(Presenter designated by underlined author.)

Plenary Lecture Abstracts

PL1

The Cancer Epigenome. Jones P. USC Norris Cancer Center, Los Angeles, CA, United States.

Epigenetic processes are reinforced by interactions between covalent chromatin marks such as DNA methylation, histone modifications and variants. These marks ultimately specify the locations of nucleosomes particularly with respect to transcriptional start sites and in regulatory regions. Understanding how the epigenome functions, therefore requires a coordinated approach so that the mechanisms by which the chemical modifications interact with nucleosomal remodeling machines are achieved to ensure epigenetic inheritance and control of gene expression. We have developed a new methodology to simultaneously map nucleosomal positioning and DNA methylation on individual molecules of DNA. We used this nucleosomal mapping technology to ascertain alterations in nucleosomal positioning during the abnormal silencing of genes by promoter hypermethylation. These experiments show that the methylation of CpG islands at the transcriptional start sites of key tumor suppressor genes results in the stable placement of nucleosomes at the transcription start site. Inhibition of DNA methylation by 5-azanucleoside treatment results in an immediate inhibition of DNA methylation and a sequence of downstream events which ultimately result in the eviction of the nucleosomes from the transcription start site and the activation of gene expression.

PL2

DNA Damage and Repair in Cancer: From Basic Science to Anti-Cancer Treatments. Helleday T[1,2,3]. [1]Stockholm University, Stockholm, Sweden, [2]University of Oxford, Oxford, United Kingdom, [3]Science for Life Laboratory, Stockholm, Sweden.

Cancers are caused by sequential gene mutations. High loads of intrinsic DNA damage in cancer cells mediate the formation of such mutations. Here, nine cancer stress phenotypes are discussed: inflammatory, microenvironmental, mitotic, proteotoxic, metabolic, oxidative, replicative, DNA damage and telomeric stress. Many stress phenotypes generate cancer-specific DNA damage, creating an optimal DNA damage load for cancer clonal evolution. DNA repair is highly important to suppress the DNA damage and is often inactivated in cancer through mutations or epigenetic silencing. Backup DNA repair pathways can become more important when the primary DNA repair pathways are lost. Inhibition of such backup pathways can be used as a synthetic lethal approach to selectively kill cancer cells. One example of this is the use of PARP inhibitors in BRCA2 tumours, where spontaneous lesions underlie the toxicity producing the synthetic lethality. DNA lesions produced at replication forks, for instance by many anti-cancer drugs, are significant substrates for homologous recombination (HR) repair. Here, different RNAi library screens using RAD51 foci formation and HR activity as end-points are presented, as well as our strategy to identify HR inhibitors that can improve anti-cancer treatments. Other approaches to selectively augment cancer-specific DNA lesions are also presented, strategies that may become important to combat cancer in the future.

PL3

Unraveling the Mystery of a Global Environmental Disease. Grollman AP. Stony Brook University, Stony Brook, NY, United States.

Aristolochic acid, a powerful nephrotoxin and human carcinogen, was shown recently to be the causative agent of Balkan endemic nephropathy. This devastating environmental disease is associated strongly with carcinomas of the upper urinary tract (UUC). Based on the widespread use of *Aristolochia* in traditional herbal remedies, we posited that aristolochic acid nephropathy (AAN) and UUC represent a long-overlooked iatrogenic disease and an international public health problem of considerable magnitude. Pursuing this hypothesis, we conducted a molecular epidemiologic study in Taiwan, where a significant fraction of the population used herbal remedies containing AA and the incidence of UUC is the highest in the world. AA reacts with DNA to form aristolactam (AL)-DNA adducts that serve as biomarkers of internal exposure to AA. In turn, these lesions generate a TP53 mutation spectrum dominated by A:T to T:A transversions located almost exclusively on the non-transcribed DNA strand. Strand bias reflects a failure to excise AL-DNA adducts by global genomic repair, accounting for the remarkable persistence of these adducts in the renal cortex. The signature TP53 mutation serves as a biomarker of the carcinogenic effect of AA. Thus, AA joins aflatoxin and vinyl chloride as one of the few human carcinogens with a TP53 mutation signature. Public health authorities in countries where *Aristolochia* has been used should be encouraged to initiate screening programs to detect AAN/UUC and to implement measures to reduce human exposure to this nephrotoxic and carcinogenic herb. (Supported by NIEHS.)

PL4

Mechanistic Basis of Resistance to PCBs and Dioxin in Atlantic Tomcod from the Hudson River. Wirgin I[1], Roy NK[1], Loftus M[1], Chambers RC[2], Franks DG[3], Hahn ME[3]. [1]Department of Environmental Medicine, NYU School of Medicine, Tuxedo, NY, United States, [2]Northeast Fisheries Science Center, NOAA Fisheries Service, Highlands, NJ, United States, [3]Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA, United States.

We investigated the molecular basis of resistance of early life-stages of Atlantic tomcod from the Hudson River (HR) to coplanar PCBs and TCDD. Most early life-stage toxicities from exposure to these compounds in fishes are mediated by the aryl hydrocarbon receptor (AHR) pathway. Tomcod from the HR exhibited two non-synonymous genetic polymorphisms (one six base deletion and one SNP) in AHR2 (the predominant form of AHR in fishes) that were nearly absent in tomcod from six other Atlantic Coast estuaries including the two in closest proximity to the HR. The variant AHR2 proteins were *in vitro* expressed and compared for their functional significance. In ligand binding assays, the HR-specific AHR2-1 protein was impaired compared to the more common AHR2-2 protein in binding TCDD. The HR AHR2-1 protein was also less able to drive reporter gene expression in transient transfection assays in AHR deficient cells treated with TCDD or PCB126 than the more common AHR2-2 protein. Additional transfections with recombinant AHR2s demonstrated that the six-base deletion in AHR2 was the mechanistic basis of resistance. We are now attempting to chronicle the timing of the onset of resistance in the HR population using tomcod archived in museum collections. Our results show that the HR tomcod population has undergone rapid evolutionary change probably due to contaminant exposure. This is the first demonstration of the mechanistic basis of resistance to contaminants in a vertebrate population and shows that rapid evolutionary change can result from one variant in a single gene.

Public Lecture Abstract

L1

Cancer in Beluga Whales from the St. Lawrence Estuary, Quebec, Canada: A Case of "One Health, One Medicine". Martineau D[1], Lair S[2]. [1]Département de Pathologie et Microbiologie, Université de Montréal, Saint-Hyacinthe, QC, Canada, [2]Département de Sciences Cliniques, Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, QC, Canada.

Of 423 beluga reported dead in the St. Lawrence Estuary (SLE) since 1982, about half (193) were examined. Cancer was the primary cause of death in 20 % of adults (n=30). This prevalence, almost unique among mammals, is about ten times higher than that reported in free-ranging mammals. The opening of the Saguenay River into the SLE is at the heart of the SLE beluga habitat. As early as 1926, a major aluminum smelter started to release massive amounts of polycyclic aromatic hydrocarbons (PAHs) into the atmosphere and water of that river, 180 km upstream and upwind of its mouth. This resulted in the heavy contamination of the sediments and the worms living in them (and upon which beluga feed) and in the contamination of indigenous fish and mammals, including beluga whales. Aluminum workers in that area suffer high rates of lung and urinary bladder cancers epidemiologically related with PAH exposure, and for which workers are now financially compensated. The concentrations of 1-hydroxypyrene (1-OHP), a PAH metabolite, are 2 to 2.5 times higher in persons living close to the smelters than in those living far. Biological exposure to PAHs has also been measured in animals - woodchucks, benthic worms, mussels and fish - inhabiting the area. Oddly, no biomarker other than cancer rate has been used in the workers to measure PAH exposure. With sufficient resources, similar biomarkers could be measured in and compared between SLE and presumably unexposed Arctic beluga to further support the role of PAH in the cancers that affect SLE beluga.

Symposium Abstracts

Symposium 1–Epigenetics: DNA Modifications and Repair

S1

DNA Methylation in Mammalian Development. Trasler JM[1,2]. [1]Research Institute at the Montréal Children's Hospital of the McGill University Health Center, Montréal, QC, Canada, [2]Depts. of Pediatrics, Human Genetics and Pharmacology & Therapeutics, McGill University, Montréal, QC, Canada.

DNA methylation is a well characterized epigenetic modulator with essential functions in the germline and embryo as well as in genomic imprinting. Most methylation in the mammalian genome occurs within CpG dinucleotides at roughly 20-30 million sites. Methylation of DNA is catalyzed by a family of DNA (cytosine-5)-methyltransferases (DNMTs) and is associated with gene silencing, especially when cytosines in the promoter region of genes are methylated. Abnormalities in DNA methylation are associated with carcinogenesis as well as perturbations in growth, placental function, and neurobehavioral processes. Following erasure in primordial germ cells, DNA methylation patterns are acquired in the germline, starting before birth in the male and during the oocyte growth phase in the female. The fact that the methylation of imprinted genes is established at different developmental times in the parental germlines suggests that male and female germ cells may be susceptible to conditions that perturb imprints at sex-specific stages of prenatal and postnatal gametogenesis. Gamete-derived methylation of imprinted genes and some single copy and repeat sequences is maintained during preimplantation development, a time when methylation across much of the genome is erased. A second wave of genome-wide methylation occurs in the peri-implantation period. Gene-targeting experiments in mice have revealed critical roles for the DNMT enzymes in establishing imprints in the germline, in meiosis in the male, in maintaining methylation imprints during preimplantation development, and in embryonic development. New insights from genome-wide methylation profiling studies will be reviewed.

S2

Role of 5-hydroxymethylcytosine in Reprogramming the Paternal Genome in the Zygote. Iqbal K[1], Jin S-G[2], Pfeifer GP[2], Szabó PE[1]. [1]Department of Molecular and Cellular Biology, Beckman Research Institute of the City of Hope, Duarte, CA, United States, [2]Department of Cancer Biology, Beckman Research Institute of the City of Hope, Duarte, CA, United States.

DNA methylation patterns are relatively stable and heritable in mammalian somatic cells, but are globally reprogrammed - erased and reestablished - between generations. Genome-wide erasure of the DNA 5-methylcytosine (5mC) patterns occurs in developing primordial germ cells and in the zygote. A rapid loss of DNA methylation takes place in the paternal pronucleus soon after fertilization in an apparently replication-independent manner. This has been suggested to involve DNA repair mechanisms. Generating double strand breaks globally would, however, jeopardize genome stability at this critical developmental stage. Here we provide evidence for an alternative mechanism of methylation-erasure in the zygote that involves enzymatic oxidation of 5mC to hydroxymethylcytosine (5hmC) by the Tet3 oxidase. We found increasing amounts of 5hmC and decreasing amounts of 5mC in the paternal pronucleus along pronuclar stages, whereas the maternal pronucleus retained 5mC and accumulated little or no 5hmC signal. Tet3, but not Tet1 or Tet2, was expressed at high levels in oocytes and zygotes. Importantly, 5hmC persisted into the cleavage-stage embryos, suggesting that 5mC oxidation is not followed immediately by global genome-wide removal of 5hmC through excision repair pathways or other mechanisms. This conclusion is supported by bisulfite sequencing data. The conversion of modified cytosines to cytosines was limited at several gene loci suggesting that a 5mC oxidation/repair cycle leading to DNA demethylation may be gene-specific.

S3

Active DNA Demethylation by Thymine DNA Glycosylase. Cortellino S[1], Xu J[1], Sannai M[1], Moore R[1], Caretti E[1], Cigliano A[1], Le Coz M[1], Devarajan K[1], Wessels A[2], Soprano D[3], Abramowitz LK[4], Bartolomei MS[4], Rambow F[5], Bassi MR[1], Bruno T[6], Fanciulli M[6], Renner C[1], Klein-Szanto AJ[1], Matsumoto Y[7], Kobi D[8], Davidson I[8], Alberti C[5], Larue L[5], Bellacosa A[1]. [1]Fox Chase Cancer Center, Philadelphia, PA, United States, [2]Medical University of South Carolina, Charleston, SC, United States, [3]Temple University School of Medicine, Philadelphia, PA, United States, [4]University of Pennsylvania School of Medicine, Philadelphia, PA, United States, [5]Institut Curie, Orsay, France, [6]Regina Elena Cancer Center, Rome, Italy, [7]University of New Mexico Cancer Research Facility, Albuquerque, NM, United States, [8]Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France.

DNA methylation is a major epigenetic mechanism for gene silencing. Whereas methyltransferases mediate cytosine methylation, it is less clear how unmethylated regions in mammalian genomes are protected from *de novo* methylation and whether an active demethylating activity is involved in gene activation. Here we show that either knockout or catalytic inactivation of the DNA repair enzyme Thymine DNA Glycosylase (TDG) leads to embryonic lethality in mice. TDG is necessary for recruiting p300 to retinoic acid (RA)-regulated promoters, protection of CpG islands from hypermethylation, and active demethylation of tissue-specific, developmentally- and hormonally-regulated promoters and enhancers. TDG interacts with the deaminase AID and the damage-response protein GADD45a. These findings highlight a dual role for TDG in promoting proper epigenetic states during development and suggest a two-step mechanism for DNA demethylation in mammals, whereby 5-methylcytosine and 5-hydroxymethylcytosine are first deaminated by AID to thymine and 5-hydroxymethyluracil, respectively, followed by TDG-mediated thymine and 5-hydroxymethyluracil excision repair.

S4

Prenatal Exposures to Environmental Pollutants and Epigenetics in Children. Holland N, Yosefi P, Aguilar R, Quach H, Huen K, Bradman A, Venkat S, Barcellos L, Eskenazi B. University of California, Berkeley, Berkeley, CA, United States.

Epigenetic changes resulting from prenatal toxicant exposures may be a biologically plausible mechanism explaining fetal origins of human diseases. The CHAMACOS longitudinal birth cohort study investigates pesticide and other environmental exposures and their effects on growth and neurodevelopment of children from low-income Mexican-American farmworker families in California; children were followed from birth to 9 years of age. First, we performed pyrosequencing of Alu and LINE-1 repeats to estimate global methylation in CHAMACOS newborns and the same children at 9 years of age (N=168). Next, we used Infinium Illumina 450K BeadChips to evaluate 485,577 CpG sites including promoters for most of the known genes, coding regions, non-coding RNAs *etc.* Levels of global DNA methylation were dependent on gender and age of children but the results of three assays (450K, Alu & LINE-1) were not correlated possibly because they characterize different parts of methylome. In the analyses of site-specific data from 450K BeadChips we identified over 2,500 CpG sites that were differentially methylated by age and >70 CpG sites displaying sex-specific differences (controlling for the False Discovery Rate). The relationship between epigenetic changes with genotypes (*PON1*) previously associated with susceptibility to pesticides and health outcomes (*Huen 2009, Eskenazi 2010*) indicates possible allele-specific differences in DNA methylation consistent with variation in gene expression. These preliminary data suggest that changes in site-specific DNA methylation, can be age, sex, and allele-specific, and may be associated with children's health and development. Supported by NIEHS and EPA grants.

S5

Genomic and Epigenomic Alterations in Cells Exhibiting Radiation-Induced Genomic Instability. Ding D, Aypar U, Tiper I, Goetz W, Baulch JE. University of Maryland, Baltimore, MD, United States.

The mechanisms of genomic instability perpetuation remain unclear. Radiation-induced mutations, double-strand breaks, or changes in gene expression alone do not account for the unstable phenotype. This study evaluates genomic and epigenetic changes in four cell lines exhibiting radiation-induced genomic instability. Analysis of miRNA-mediated changes in gene expression showed little evidence for miRNA mechanisms in the instability exhibited by these clones. However, changes were observed in LINE-1 and alu repeat element DNA methylation using combined bisulfite restriction analysis, and analysis of global methylation by arbitrary priming methylation sensitive PCR indicated that both DNA hypo- and hypermethylation may be observed in irradiated clones. However, results of specific locus methylation sensitive PCR (MSP) demonstrate no effect of irradiation or genomic instability on promoter methylation for tslc1 or cdh1 genes. MSP for the NFkB promoter showed no amplification for two unstable clones, suggesting a deletion/mutation event in the human NFkB promoter. Sequencing indicated that a novel transposable element in the promoter region may facilitate translocation of the hNFkB gene. Unstable clones evaluated by RT-PCR for highly conserved regions of NFkB mRNA also demonstrated melting curves that differed from the parental cell line. Sequence data supports the hypothesis that an alternate NFkB transcript is expressed. Ongoing analyses will characterize this alternate transcript and its functionality, and evaluate the role for transposable elements in translocation of hNFkB in unstable cells. Implications of these findings may extend to mechanisms of NFkB over-expression and contribution to carcinogenesis and inflammatory diseases. [This work supported by NASA grants NNX07AT42G, NNJ06HD31G.]

Symposium 2–Application of Toxicogenomics in Regulatory Decision-Making

S6

Challenges to the Use of Emerging Technologies in Regulatory Risk Assessment. Hughes K. Health Canada, Ottawa, ON, Canada.

The field of toxicogenomics and other emerging technologies is advancing at an incredible rate. There has been an explosion in the amount and type of information being generated on the health and environmental effects of chemical substances. However, although the rate of development on the research side has been extremely rapid, progress on the part of regulatory agencies to incorporate these data into risk assessment has been much slower. There are a number of challenges and obstacles that must first be overcome before toxicogenomics data can be fully integrated into risk assessment processes on a regular basis. These challenges include but also go beyond simply better understanding and communicating the science and relate to all levels of the regulatory environment, including risk assessors, risk managers as well as senior management and administrators of risk assessment programs. Overcoming these challenges will require focussing and coordinating efforts in areas of research designed to increase confidence in the technology and advancing the dialogue between researchers and regulators to facilitate development of methodology for incorporation of toxicogenomics in mechanism based risk assessments.

S7

Application of Toxicogenomics in Health Risk Assessment. Waters MD. ILS, Inc, Research Triangle Park, NC, United States.

Toxicogenomics (TGx) has a potentially pivotal role in health risk assessment. Events of the past year have dramatically changed the landscape of TGx in the public domain. The DrugMatrix database was purchased by the National Toxicology Program (NTP) in October, 2010, and is a now a source of standardized acute dose-response (D-R) TGx studies on 659 drugs and chemicals. The Japanese TG-GATEs database contains similar data on 131 compounds and the two databases have 82 compounds in common. With approximately 740 TGx studies and 560 NTP rodent cancer bioassays available (60 chemicals in common), there is now plenty of data to integrate for health risk assessment. In reviewing the literature, it is clear that the majority of published TGx studies relate to carcinogenicity and hepatotoxicity (Waters, *et al.*, 2010, *Mutat Res* 705, 184-200). Nine laboratories have validated predictive mode of action (MOA) signatures in rodent model systems over the past 7 years. Nesnow, *et al.*, EPA-HQ-OPP-2011-0284-0006, recently demonstrated the integration of apical and TGx data (Nesnow, *et al.*, 2009, *Toxicol Sci* 110, 68-83) to formally derive a novel MOA for propiconazole, previously thought to behave like phenobarbital. Thomas, *et al.*, 2011, *Toxicol Sci* 120, 194-205, have described the application of transcriptional benchmark dose values in quantitative cancer and noncancer risk assessment. We will discuss an approach to the integration of public domain datasets from TGx and NTP cancer bioassays, beginning with the examination of previously reported NTP toxicology and histopathology from 14-day, 90-day and 2-year studies.

S8

Comparison of Transcriptional Benchmark Doses to Traditional Cancer and Non-Cancer Endpoints for Benzo(a)pyrene. Yauk C, Moffat I, Nong A, Kuo B, Labib S, Williams A, Halappanavar S, Lemieux C, White P. Health Canada, Ottawa, ON, Canada.

This study aimed to determine if genomics data can inform risk assessments more quickly, comprehensively and cost effectively than traditional approaches. Benzo(a)pyrene (BaP) was selected since it is present in the environment (99% of exposure by ingestion), regulatory agencies use BaP as an indicator for mixtures of polycyclic aromatic hydrocarbons, and BaP has a well characterized mode of action in animals for cancer, causing DNA damage through reactive metabolites, leading to mutation and tumor formation. Dose-response experiments were conducted following acute (3 day) and sub-chronic (28 day) exposure to BaP by gavage in male mice. Organ weight, as well as DNA adducts and mutations in liver and lung were evaluated, and used to calculate benchmark doses (BMDs). Hepatic and pulmonary gene expression profiles were generated using DNA microarrays. Dose-related changes in gene expression were used to calculate transcriptional BMDs for statistically significant KEGG pathways. The $BMDL_{10}$ for liver DNA adducts was 14 mg/kg/day and transcriptionally-derived cancer and non-cancer pathway $BMDL_{10}$s ranged from 5-125 mg/kg/day (average slope factor 0.24 $(mg/kg/day)^{-1}$). The most recent risk assessment reports a point of departure (POD) of 5 mg/kg/day for non-cancer effects and a cancer slope factor of 1.7 $(mg/kg/day)^{-1}$ (California EPA, 2010). This dataset is being integrated into a published systems biology model to evaluate the dose-response curve related to AhR/p53 pathways. Comparison of this dataset to published data from human cells supports the biological relevance of cross-species extrapolation of genomics data. Thus, transcriptional changes may provide estimates of the PODs for human risk assessment.

S9

Opportunities for Integrating Toxicogenomic Approaches for Regulatory Decision Making. Faustman EM, Yu X, Wegner SH, Harris S. University of Washington, Seattle, WA, United States.

Toxicogenomics offers both fantastic opportunities to follow the details of molecular response but it also provides many challenges as we look to this very dynamic tool to answer specific toxicology and pathology questions. Integration of signal using a systems based approach provides unique solutions to these challenges. This presentation will focus on three concepts for integration using several case studies on reproductive and developmental toxicology from our Institute. The first study case will look at using toxicogenomics to define "normal" response across development. Examples from both male and female reproduction will be used. Second, toxicogenomic approaches for working across platform and cross level of biological organization will be presented using examples of *in vitro* to *in vivo* comparisons for male reproductive toxicants. In this example we will use data from a group of 6 developmentally toxic and non toxic phthalates that were tested in an *in vitro* 3-D germ-sertoli cell culture. Third, the importance of using contextual information such as anchoring will be provided. Three approaches for quantitatively evaluation response will be discussed and critiqued. An integrated systems based framework will be presented. Overall the emphasis will be on integration of signals rather than focus on a single chemical or group of chemicals. Supported by grants CHC Center NIEHS 5P01ES009601, EPA RD-83170901, Biomarkers EPA RD-83273301, CEEH NIEHS P30 ES07033.

S10

An Approach to Using Toxicogenomic Data in Risk Assessment: Dibutyl Phthalate Case Study. Euling SY[1], White L[3], Ovacik AM[4], Makris SL[1], Sen B[3], Androulakis IP[4], Hester S[2], Gaido KW[5], Kim AS[6], Benson R[7], Wilson VS[2], Keshava C[2], Keshava N[2], Foster PM[3], Gray Jr. LE[2], Chiu WA[1], Thompson C[8]. [1]NCEA, U.S. EPA, Washington, DC, United States, [2]US EPA, Research Traingle Park, NC, United States, [3]NIEHS, Researcg Traingle Park, NC, United States, [4]ebCTC, Rutgers, UMDNJ, Piscataway, NJ, United States, [5]US FDA, Rockville, MD, United States, [6]Allergan, Inc., Irvine, CA, United States, [7]Region 8, U.S. EPA, Denver, CO, United States, [8]ToxStrategies, Inc., Katy, TX, United States.

Approaches for utilizing genomic data in risk assessment are needed. A team of scientists developed an approach for integrating genomic data in risk assessment that included examining the genomic and toxicity datasets in conjunction, defining questions to direct the evaluation, and evaluating consistency in findings. A case study of the approach was performed for dibutyl phthalate (DBP). An evaluation of the DBP toxicity dataset identified some effects that could not be explained by either reduced fetal testicular testosterone production or Insl3 gene expression, two well-established MOAs for many of the male reproductive toxicity effects. The DBP toxicogenomic data set consists of nine toxicogenomic and other gene expression (microarray, RT-PCR, protein) studies, eight of which assessed the rat testis. The testis gene and pathway effects were fairly consistent across studies. A pathway analysis of one microarray study of the testis identified additional pathways in a number of processes (*e.g.*, cell signaling, cell adhesion). These putative new pathways may be associated with DBP testis effects that are currently unexplained. The available DBP toxicogenomic dataset informed the testis mechanism of toxicity. Research needs, such as multiple dose microarray studies, were identified. Methods to analyze genomic data for risk assessment purposes were developed including a pathway analysis method. The approach and methods for utilizing toxicogenomic data in risk assessment may be used in chemical assessments and in the proposed toxicity-pathway-based risk assessment paradigm. The views expressed are those of the authors and do not represent the policies and/or endorsement of the U.S. EPA.

S11

Integration of Genomic Biomarkers in Cancer Risk Assessment Paradigm. Aubrecht J. Pfizer Inc., Groton, CT, United States.

Despite the scientific progress in understanding of carcinogenicity, experimental approaches for assessing oncogenic risk associated with exposure to chemicals relies mainly on methods originally developed in 1960- and 1970-ties. However, currently used assays do not offer sufficient mechanistic information applicable for assessing oncogenic risk to humans. Recent advances in molecular biology and bioinformatics have enabled interrogating cellular responses to chemical exposure on genomic level eventually leading to identifying molecular pathways and networks mechanistically involved in chemical carcinogenesis (system biology approach). Thus new experimental approaches such as *in vitro* cell-based toxicogenomic methods and systems biology interpretation of the data are being explored to improve chemical cancer risk assessment. Recent advances in humanized animal models indicate that evaluating molecular pathways associated with cancer development might ultimately lead to better cancer risk assessment for humans *via* studying species specific effects *in vivo*. Furthermore, the presentation will discuss emerging applications of systems biology-based approaches in chemical cancer risk assessment for regulatory decision making.

S12

Practical Considerations for the Application of Toxicogenomics to Risk Assessment: Early Experience, Current Drivers, and a Path Forward. Boverhof DR. The Dow Chemical Company, Midland, MI, United States.

Traditionally, toxicology has been an observational science that has investigated effects at cellular and organism level through various endpoint-specific assays. Toxicogenomics has created the opportunity to study treatment-related perturbations at the transcriptome level to gain new insights into the manifestation of toxicity. With this technology came the expectation of more facile and informative predictions of toxicity in shorter term assays through a better understanding of mechanisms of action. Other expectations included the ability to more effectively bridge *in vitro* and *in vivo* responses to reduce animal use and dramatically improve the ability for cross-species extrapolations. Alongside these expectations, came concerns over the ability to interpret, organize, report, and store such large amounts of data and questions were raised on the perceived increased sensitivity of the technology to detect changes of unknown toxicological significance. Although the independent contributions of toxicogenomics to risk assessment have thus far been limited, researchers are now re-evaluating the experiences of the past decade to strategically apply this technology to enhance mode of action and weight-of-evidence approaches to risk assessment. This presentation will review some of the early expectations and experiences with toxicogenomics that can be used to appropriately shape future applications. Perspectives will be shared on the importance of recognizing the strengths and weaknesses of toxicogenomics when applied to the different components of the risk assessment process. It is hoped that these experiences will lead to more focused and successful application of toxicogenomic studies such that increased contributions to chemical risk assessment will be realized.

Symposium 3–Etiology of Autism: Genetics, Epigenetics, and the Environment

S13

Overview of the Etiology of Autism. Halladay A. Autism Speaks, New York, NY, United States.

Autism is now an urgent public health issue, affecting approximately 1% of children, and 1 in 70 boys in the US. Over the past 30 years, the prevalence of autism spectrum disorders has increased over 600%. Recent research indicates that while some of this increase can be attributed to changes in diagnostic practice and improved awareness, a substantial portion of this increase remains unexplained. Therefore, a wide scope of environmental influences that potentially increase risk of autism, including sociological, toxicological and nutritional factors, need to be further studied. This is further supported by the heterogeneity in the symptoms of autism, where many causes may lead to many different disease phenotypes. A large effort has been dedicated to identifying and characterizing the genetic risk factors associated with autism, with relatively little attention directed at the role of environmental factors, and their interactions with genetic susceptibility. Many autism risk genes have been demonstrated to be regulated epigenetically, suggesting that environmental factors such as chemicals and diet may influence risk of the disease across generations. Identification of these and other unknown risk factors will lead to targeted prevention and intervention strategies where some exposures may be avoided so that the effects can be mitigated. This presentation will provide a summary on genetic and environmental risk factors for ASD and research being undertaken to address major questions in this field.

S14

Etiology of Autism: A Role for Epigenetics? Weksberg R. Genetics and Genome Biology, Research Institute, The Hospital for Sick Children, Toronto, ON, Canada.

Autism spectrum disorders (ASD) are a group of childhood neurodevelopmental disorders involving problems in social interaction, communication, and repetitive behaviors. The etiology of ASD is complex and multifactorial. Several lines of evidence implicate epigenetic alterations as one of the multifactorial causes of autism. With a view to identifying epigenetic alterations associated with ASD, we initiated genome-wide DNA methylation analyses in clinically accessible tissues such as blood or saliva of several types of ASD patients. We are studying genetic syndromes associated with an increased frequency of autistic features that are caused by mutations in genes encoding proteins that function in epigenetic regulation. We are also screening ASD cases with a history of subfertility/medically assisted conception since animal models suggest that such treatments can lead to increased rates of DNA methylation alterations. Finally, we are performing locus-specific and genome-wide DNA methylation analyses in idiopathic ASD cases, *i.e.* with no known genetic or environmental risk factors. Candidate genes identified by these approaches will be discussed.

S15

Genomic Rearrangements in Autism: The Contribution of Copy Number Loss and Gain to the Etiology of Autism Spectrum Disorders. Gregory SG. Duke Center for Human Genetics, DUMC, Durham, NC, United States.

Autism comprises a spectrum of behavioral and cognitive disturbances of childhood development. The core autism phenotype includes deficits in social interaction, language development and patterns of repetitive behaviors and/or restricted interests. Although Autism Spectrum Disorders (ASDs) are known to be highly heritable, less than 10% of autism cases have been attributed to an underlying genetic component. Numerous approaches including genetic linkage, genome-wide and candidate gene association, and gene expression analysis have been used to identify the 'missing heritability' implicated in the development of autism. However, the heterogeneous nature of ASDs has limited these approaches. An alternative method is to characterize copy number variants (CNVs), that is, chromosomal deletions and duplications, which are known to be present within at least 5% of individuals with idiopathic autism. Autism CNVs have been shown to involve almost all chromosomes, with the most frequent alterations localizing to chromosome 15q11-13. In this presentation I will discuss a number of different methods that have been used to characterize autism related CNVs, including traditional cytogenetic analyses, more recent large insert bacterial, single nucleotide polymorphism, oligonucleotide microarrays, and the impact that next generation sequencing approaches will have on the identification genomic imbalances implicated in the development of ASDs.

S16

The Epigenetic Interface of Genetic and Environmental Risk Factors in the Etiology of Autism. LaSalle JM[1], Woods R[1], Vallero R[1], Golub M[1], Suarez J[1], Kostyniak PJ[2], Pessah IN[1], Berman R[1]. [1]University of California, Davis, Davis, CA, United States, [2]University at Buffalo, Buffalo, NY, United States.

The widespread use of polybrominated diphenyl ethers (PBDEs) as commercial flame retardants has raised concern about potential effects on the developing brain, particularly in genetically susceptible individuals. Autism spectrum disorders are increasingly common and susceptibility is mediated by both genetic and environmental factors. Epigenetic mechanisms act at the interface of genetic and environmental risk factors for complex disorders and epigenetic pathways are critical for human neurodevelopment. Rett syndrome (RTT) is an X-linked neurodevelopmental disorder caused by mutations in MECP2, encoding epigenetic factor methyl-CpG-binding protein 2 (MeCP2). A truncation mutant mouse model ($Mecp2^{308/y}$) with social behavioral defects is a useful mouse model for examining environmental modifying factors. To test potential genetic, epigenetic, and environmental interactions relevant to social and cognitive behaviors, a daily perinatal low-dose BDE-47 exposure was performed on $Mecp2^{308/+}$ dams bred to wild-type C57Bl/6J males. Perinatal BDE-47 exposure negatively impacted fertility of $Mecp2^{308/+}$ dams and preweaning weights of female pups. Global hypomethylation of brain DNA was observed specifically in BDE-47 exposed female offspring and correlated with reduced sociability in a genotype-independent manner. A reversing interaction of Mecp2 genotype on BDE-47 exposure was observed in a short-term memory test of social novelty that correlated with increased Dnmt3a levels specifically in BDE-47 exposed $Mecp2^{308/+}$ mice. In contrast, a compounding BDE-47*Mecp2 interaction was observed in a test of spatial learning and long-term memory. These results suggest that genetic and environmental interactions on the developing brain are complex and involve sexual dimorphism, epigenetic dysregulation, compensatory molecular mechanisms, and specific behavioral deficits.

Symposium 4–Genotoxic Impurities: The Current Environment and Future Possibilities

S17

Mutagenic Impurities: Introduction to the Current Environment and On-Going. De Antonis DM. Pfizer Inc., Groton, CT, United States.

Assessing and controlling the risk of mutagenic impurities in pharmaceutical products continues to be an area of high attention and activity within the regulatory and industrial environments. The regulatory landscape has evolved significantly over the past decade and continues at present as part of the ICH process. Similarly, industry approaches and expertise in this area continues to grow significantly, both in terms of assessing risk and in development of robust process control options. This presentation will provide an overview of the current regulatory and industrial landscape and future direction.

S18

Case Studies Illustrating Validation of *In Silico* Prediction of Chemicals for Mutagenicity and Calculation of Compound-Specific Threshold of Toxicological Concern (TTC). Reddy MV, McGettigan KK, Gealy R, Kreatsoulas C, Galloway SM. Merck & Co., Inc., West Point, PA, United States.

A step-wise approach to *in silico* analysis of chemical structures for their potential mutagenicity will be described. The approach provides 97% or better in accuracy for predicting the negative outcomes in the Ames test for mutagenicity for process intermediates and reagents used in the synthesis of drug substances. Case studies will be presented to demonstrate various aspects of *in silico* analysis and the subsequent steps in assessing acceptable levels of exposure to the alerting molecules. These include: (i) validation of a prediction either as acceptable or dismissible based on the learning set structures and knowledgebase, (ii) justification for >1.5 mg Threshold of Toxicological Concern (TTC) based on the exposure levels to naturally-occurring compounds containing the same alert; and (iii) justification for >1.5 mg TTC based on known carcinogenic potency of structurally similar compounds.

S19

Dose-Response Relations: The Impact of Non-Linearity. Guerard M, Singer T, Gocke E. F. Hoffmann-La Roche Ltd., Basel, Switzerland.

As pointed out in Kroes *et al*. (2004) and the EMEA guideline on the limits of genotoxic impurities (2006) the calculation of the generic TTC value encompasses a multitude of conservative (worst case) assumptions. Among these the "low dose linearity" concept for genotoxic carcinogens is possibly the most conservative presumption as demonstrated in two recently published studies: a) For the exemplary alkylating agent ethylmethane sulphonate (EMS) the dose-response relations were shown to be distinctly thresholded. Therefore, the carcinogenic dose-response of EMS must also be thresholded. b) For the potent genotoxin dibenzo[a,l]pyrene (IARC class 2B) a clearly sublinear dose-response was demonstrated in a "mega cancer" study in the rainbow trout (Bailey, 2009). Based on this data we estimate the factor by which the 'linear dose-response concept' overestimates the "true" cancer incidence in the very low dose region. References: Kroes *et al.* (2004) Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet; *EMEA* (2006) Guideline on the limits of genotoxic impurities; Gocke *et al.* (2009) *In vivo* studies in the mouse to define a threshold for the genotoxicity of EMS and ENU. *Mutat Res*. 2009 Aug 2;678(2):101-7. Epub 2009 Apr 17.; and Bailey *et al.* (2009) Nonlinear Cancer Response at Ultralow Dose: A 40800-Animal ED001 Tumor and Biomarker Study. *Chem. Res. Toxicol*. 2009, 22, 1264-1276.

S20

Application of the *In Vivo* Pig-a Mutagenicity Assay for Assessing Dose-Response Relationships. Dertinger SD. Litron Laboratories, Rochester, NY, United States.

In vivo Pig-a gene mutation assays based on flow cytometric detection and enumeration of erythrocytes and reticulocytes that lack cell surface CD59 expression have been previously reported. A second-generation scoring procedure will be described that utilizes immunomagnetic separation of wild-type and Pig-a mutant cells in conjunction with flow cytometry to dramatically increase the rate at which mutant cell frequencies are acquired. The new methodology was initially evaluated *via* reconstruction experiments, and subsequently with rat studies that involved low dose administrations of 1,3-propane sultone or melphalan to Sprague Dawley rats. These results, together with power analyses, indicate that the use of immunomagnetic separation technology greatly enhances the assay's ability to detect modest changes to mutant cell frequency. This can be attributed to the new found ability to interrogate ≥ 3 million RETs and ≥ 100 million RBCs per sample in less than 7 minutes. The feasibility of using this improved analytical platform to quantitatively investigate *in vivo* dose-response relationships will be discussed.

Symposium 5–Mechanisms and Roles of PARP in Response to Environmental Genotoxins

S21

Programming of DNA Damage Signaling and Repair by Poly(ADP-ribose). Poirier GG[1], Gagne J-P[1], Pic E[1], Rouleau M[1], Krietsch J[1], Isabelle M[1], Masson J-Y[1], Hendzel M[2]. [1]CHUQ Research Center, Laval University, Quebec, QC, Canada, [2]Cross Cancer Institute, University of Alberta, Edmonton, AB, Canada.

Poly(ADP-ribose) (PAR) synthesis occurs during DNA damage because of the induction of single and double strand breaks. The cellular PAR concentration can increase by at least 100-fold and this leads to signaling events by PAR-binding proteins. These proteins are involved in HR (Homologous recombination) and NHEJ (Non homologous recombination). These proteins such as ATM MRE11 are recruited to DNA damage laser tracts within seconds because of the rapid recruitment of PARP-1 and 2. Inhibition of PAR synthesis results in a delayed recruitment to DNA strand breaks. The mechanisms by which PARP inhibitors work in DNA repair will be discussed. Most of the proteins are recruited as protein complexes bound to one protein that is a polymer binding domain. Quantitative proteomics will be applied to study the dynamics of protein binding proteins during DNA damage signaling. Our approach to better decifer protein complexes that interact with PAR and perform quantitative proteomics will be described. We use bioinformatics tools combined with high throughput peptide synthesis. These interactors are validated in intact cells by using laser tracks with 2-photon confocal microscopy and I-SCE-I confocal colocalization. The potential of PARP inhibitors in cancer treatment involving defects in homologous and non homologous end rejoining will be discussed. Supported by CIHR.

S22

Targeted Therapy of Breast Cancer Using PARP Inhibitors. Ford JM. Stanford University School of Medicine, Stanford, CA, United States.

Triple-negative breast cancers (TNBC), defined pathologically by the lack of expression of estrogen and progesterone receptors and the lack of overexpression or amplification of the HER2/neu oncogene, account for approximately 15% of breast cancers overall. TNBCs have an aggressive clinical course, and poorer disease-specific survival than with hormone receptor-positive subtypes. Women that inherit mutations in one allele of the BRCA1 breast cancer susceptibility gene are at significantly elevated risk for developing TNBC. Unlike for patients with hormone receptor-positive or HER2-positive breast cancer, specific therapeutic targeting of the triple-negative subtype has been limited due to the absence of an identifiable target. Many effective cytotoxic chemotherapies exert their anti-tumor effect through production of DNA damage and efficient cellular DNA repair can mitigate this injury resulting in treatment resistance. Breast tumors arising in patients with a germline BRCA1 mutation are associated with reduced DNA repair capacity due to the loss of BRCA1 function and are hypothesized to rely more heavily on alternate compensatory DNA repair processes for survival. BRCA1 and BRCA2-deficient cells have been found to be markedly sensitive to inhibition of poly(ADP-ribose) polymerase (PARP), in contrast to those cells wild-type or heterozygous for BRCA1 or BRCA2. PARP is required for base-excision repair and if inhibited, repair-associated breaks result in replication fork-mediated double-strand break formation, which require BRCA1- and BRCA2-associated recombination to resolve. Inhibition of PARP is thought to enhance the effect of DNA damage; consequently, it is hypothesized that small-molecule inhibitors of PARP will have a role in the treatment of cancer, both alone and in combination with DNA damaging agents, particularly in tumors with defects in DNA repair pathways leading to a chemical “synthetic lethality”. Early reports demonstrating efficacy of PARP inhibitors in women with advanced BRCA-associated and TNBC have been reported. Responses seen in both triple-negative and non-triple-negative patients with BRCA mutations suggest that the selection of patients based on shared DNA repair defects trumps phenotypic subtype and has important implications for future investigations.

S23

NAD^+-Dependent Gene Regulation by PARP-1. Kraus WL. University of Texas Southwestern Medical Center, Dallas, TX, United States.

Nuclear NAD^+ synthesis and catabolism generates signals that regulate the composition, structure, and function of chromatin, leading to changes in patterns of gene expression. The nuclear NAD^+ biosynthetic enzyme nicotinamide mononucleotide adenylyltransferase-1 (NMNAT-1) contributes to transcriptional regulation by generating NAD^+. The NAD^+ is used by nuclear enzymes, such as poly(ADP-ribose) polymerase-1 (PARP-1), in chemical reactions that modify chromatin. PARP-1 is a nucleosome-binding protein that catalyzes the NAD^+-dependent addition of poly(ADP-ribose) (PAR) to itself and other nuclear chromatin-associated enzymes as a means of regulating its chromatin-dependent activities. We have explored the role of nuclear NAD^+ signaling in the regulation of chromatin-dependent outcomes by PARP-1 by using a variety of biochemical and genomic approaches. For example, in expression microarray analyses, we have identified genes that are co-regulated by NMNAT-1 and PARP-1. In ChIP-chip-based genomic localization analyses, we have found that PARP-1 binds in peaks near the transcriptional start sites of many genes in patterns that are negatively correlated with the binding of H1, another nucleosome-binding protein. In gene-specific cell-based assays, we have found that PARP-1 controls histone methylation patterns, nucleosome positioning, and the recruitment of the basal transcription machinery to target promoters. Interestingly, PARP-1 recruits NMNAT-1 to the promoters of target genes to support the NAD^+ requirement of its enzymatic activity. Collectively, our results point to a complex and highly integrated pathway for nuclear NAD^+ signaling that regulates chromatin composition, structure, and function.

S24

Analysis of the NAD Metabolome in Response to DNA Alkylation. Goellner EM[1], Trammell S[2], Sobol RW[1], Brenner C[2]. [1]University of Pittsburgh Cancer Institute, Pittsburgh, PA, United States, [2]University of Iowa, Iowa City, IA, United States.

Methyl-N′-nitro-N′-nitrosoguanidine (MNNG) is a DNA alkylating agent that modifies the O6 of guanine and the O4 of thymine, necessitating base excision repair (BER). Recently, Tang and co-workers (Mol Cancer Res 8, p. 67–79, 2010) showed that poly ADPribose polymerase 1 (PARP1) functions as a sensor for BER failure that induces caspase-independent cell death. Because the data indicated that cell death is synergistic with inhibition of NAD biosynthesis, we endeavored to characterize the NAD metabolome as a function of MNNG treatment in glioblastoma-derived cell line LN428. Here we will use LC-MS to characterize time-dependent and DNA-damage dependent changes to the NAD metabolome.

S25

Role of PARP Inhibitors in ATM Deficient Human Malignancies. Williamson CT[1], Muzik H[2], Dobbs TA[1], Kubota E[1,2], Elegbede A[2], Bebb DG[2], Lees-Miller SP[1,2]. [1]Department of Biochemistry and Molecular Biology, University of Calgary, Calgary, AB, Canada, [2]Department of Oncology, Tom Baker Cancer Centre, University of Calgary, Calgary, AB, Canada.

Small molecule inhibitors of PARP have shown promise in the treatment of malignancies deficient in BRCA1 and BRCA2 and preclinical studies have demonstrated the potential utility of PARP inhibitors in targeting cells deficient in ATM. Up to 50% of Mantle Cell Lymphoma (MCL) cells are deficient in ATM, and we previously showed that ATM-deficient MCL cell lines are more sensitive to PARP inhibitors than their ATM proficient counterparts (Williamson *et al.*, *Molecular Cancer Therapeutics*, 9, 347-357, 2010). Here we report that MCL cell lines that lack functional ATM and p53 are more sensitive to the PARP inhibitor olaparib than cells lacking ATM function alone, both *in vitro* (cell lines) and *in vivo* (murine xenograft models). In addition, inhibition of ATM in a p53 mutant background enhanced olaparib sensitivity in MCL cells, with implications for other p53 defective tumours. Moreover, olaparib induced DNA-PK-dependent phosphorylation and stabilization of p53 in ATM-deficient MCL cells, with expression of p53-responsive cell cycle checkpoint regulators but surprisingly direct inhibition of DNA-PK reduced the toxicity of olaparib in ATM-deficient cells. We conclude that both DNA-PK and p53 play critical roles in the response of ATM-deficient MCL cells to olaparib and the combination of ATM and PARP inhibitors may be useful in the treatment of p53-deficient malignancies. We have also identified several human gastric cancer and lung cancer cell lines that have low levels of ATM expression and work is in progress to characterize their response to PARP inhibitors *in vitro* and *in vivo*.

S26

Temporal and Spatial Resolution of PARP Activation-Induced Cellular Energy Modulation. Goellner EM[1,2], Johnson AL[2], de Moura MB[1,2], Wheeler DS[1], Brown AR[2], Wang X-H[2], Van Houten B[1,2], Romero G[1], Sobol RW[1,2]. [1]Department of Pharmacology & Chemical Biology, University of Pittsburgh, Pittsburgh, PA, United States, [2]University of Pittsburgh Cancer Institute, Pittsburgh, PA, United States.

Alkylating agents in the environment or used in the clinic can induce multiple types of DNA damage, much of which is repaired by the base excision repair (BER) pathway. Defects in or inhibition of select BER genes/proteins triggers BER failure and the formation of excess BER intermediates such as abasic sites or strand-breaks containing a 5'deoxy-ribose phosphate moiety. Accumulation these repair intermediates leads to nuclear PARP1 hyperactivation and necrotic cell death from a rapid depletion of cellular NAD^+ and ATP. Alkylating agent-induced DNA damage, DNA base lesion processing and the resulting PARP1 hyperactivation is nuclear whereas NAD^+ and ATP pools are separated in discrete sub-cellular locations, including the nucleus, the cytosol and the mitochondria, raising the question of whether nuclear PARP1 hyperactivation impacts the metabolite pools outside of the nucleus. To address this question, we used sub-cellular imaging and functional analyses to determine if nuclear PARP1 hyperactivation impacts the cytosolic, mitochondrial and/or nuclear ATP pools equally and at the same rate following DNA damage. Further, we utilized real-time functional analyses to determine the impact of DNA damage, NAD^+ depletion and/or PARP1 hyperactivation on mitochondrial function. Overall, these studies will address the impact of BER capacity on DNA damage induced changes in metabolism, using real-time techniques to uncover the temporal and spatial resolution of PARP1 activation-induced cellular energy modulation.

Symposium 6–Environmental Exposures' Impact on Male Germ Cells and Consequences to the Embryo: Is the Predominant Damage Genetic/Epigenetic?

S27

Impact of Paternal Drug Exposures on Progeny Outcome: The Roles of Genetic *versus* Epigenetic Mechanisms. Hales BF, Robaire B. McGill University, Montréal, QC, Canada.

There is increasing evidence that paternal drug exposures have an adverse impact on progeny outcome. Using a rat model we determined the consequences of paternal anticancer drug exposures on progeny. Paternal exposure to the drugs used to treat non-Hodgkin lymphoma (CHOP) increased pre- and post-implantation losses; in contrast, the drugs used for testicular cancer (BEP) did not affect litter size but increased early post-natal mortality. The developmental toxicity of paternal exposure to a single chemotherapeutic, cyclophosphamide (CPA), was germ cell phase specific. Post-implantation loss was increased most in litters sired by germ cells first exposed during sperm chromatin remodeling, coincident with the greatest susceptibility to DNA breaks. Paternal CPA exposure also disrupted epigenetic programming and DNA damage recognition/repair in embryos. In control zygotes, the male pronuclei underwent a genome-wide DNA demethylation, while the female pronuclei remained hypermethylated; in contrast, the male pronuclei in zygotes fertilized by CPA-exposed sperm were dramatically hypomethylated. Histone H4 at lysine 5 was hyperacetylated in both pronuclei in embryos sired by CPA-treated males. Phosphorylated histone H2AX was increased in a biphasic manner in the paternal genome, while poly(ADP-ribose) polymerase-1 was markedly elevated in both parental genomes in zygotes fertilized by CPA-treated males. DNA damage in the paternal genome was manifested as an increase in micronuclei in 2-cell embryos. Thus, paternal CPA exposure affected the epigenetic programming and DNA damage response in both the male and female pronuclei. Dysregulation of epigenetic programming in the zygote may contribute to heritable instabilities later in development. Supported by CIHR.

S28

Role of Histone Modifications in Male Germ Cell Development and Progeny Outcome. Kimmins S, Godmann M, Lambrot R, Chountalos G, Lafleur C, Paquet M. McGill University, Montréal, QC, Canada.

Introduction: The epigenome refers to the epigenetic state of a cell and includes post-translational modifications on the histones and methylation of DNA. Histone modifications in human and mouse sperm have been associated with developmental genes suggesting they may influence embryo development and offspring health. However to date no functional role has been assigned to histones retained in sperm. We hypothesized that histone methylation in mouse sperm influences embryo development. Methods: Using transgenic mice we examined the effects of the over-expression of the histone H3 demethylase, KDM1 on the sperm epigenome and the consequences of an altered sperm epigenome for offspring. Results: The expression of the transgene was restricted to gametes and over-expression of KDM1 beginning in spermatogonia results in sperm that has hypo-methylated histone H3 at lysine 4 and 9. Examination of 300+ pups sired by transgenics in comparison to 200+ pups sired by non-transgenics showed that 39% of offspring sired by transgenics are abnormal, and that 36% die before postnatal day 21. Analysis of offspring from transgenic lineage revealed severe abnormalities such as craniofacial defects, missing limbs, skeletal malformations, extra digits, skin and vascular defects. With each successive generation the survivability of pups was significantly reduced. Discussion: This model shows for the first time that altered methylation of histone H3 in sperm has significant effects for offspring development and survival. Given that the environment can alter the epigenome in developing sperm this study highlights underlying mechanisms of how and altered sperm epigenome could have transgenerational effects.

S29

Effects of Dietary Folic Acid Intake on Somatic and Germ Cells within and across Generations. Wade M[1], Swayne B[1], Yauk C[1], Williams A[1], Behan N[2], MacFarlane A[2]. [1]Environmental Health Science & Research Bureau, Healthy Environments and Consumer Safety Branch, Ottawa, ON, Canada, [2]Nutrition Research Division, Health Products and Food Branch, Health Canada, Ottawa, ON, Canada.

About 70% of Canadians exhibit high folic acid (FA) status due to FA supplementation. FA influences both nucleotide synthesis and DNA methylation suggesting an influence on mutation and DNA repair. The present work examines the effects of dietary FA on DNA damage in somatic and germ cells. Mice were fed FA deficient, replete or supplemented diets for 14 weeks before breeding. Pups (F1) were weaned onto replete diet. In parallel, pups from litters maintained on replete diet were weaned onto deficient or supplemented diet. Assessment of germ cell DNA damage and mutation rates will be evaluated in F1 and F3 males by sperm chromatin condensation assay and expanded simple tandem repeat instability assays and using cauda sperm. Somatic mutagenesis and clastogenesis were assessed by the rates of Piga absence (in F1 only) and micronuclei in reticulocytes /erythrocytes, respectively. Litters from deficient dams were smaller but implantations site numbers were not affected. Males weaned to deficient diet had a 1.24-fold increase ($P < 0.001$) in micronucleus frequency in while F3 males had a 2.7-fold increase ($P < 0.0001$) in micronucleus frequency in erythrocytes. Supplementation did not alter micronucleus frequencies. Piga-deficient erythrocytes were not altered by in males weaned onto any diet. Assessments of ESTR and SCSA in cauda sperm are ongoing. Our data suggest that deficiency of FA leads to an increased rate of spontaneous clastogenesis in somatic tissues with effect increasing across generations of FA deficiency. Results from our ongoing investigations on germ cells will be available at the EMS meeting.

S30

Environmental Organochlorine Exposures and Sex Chromosome Disomy in Human Sperm. Perry MJ. George Washington University, Washington, DC, United States.

Each year more than 2 million couples in the U.S. who want to have children are infertile, and over 2 million conceptions are lost before the twentieth week of gestation. About 40% of cases of human infertility are due to male factors. Errors in chromosome segregation during meiosis result in structural aberrations and imbalances in chromosome number known as aneuploidy. Many new gene mutations seen in offspring and most abnormalities in the numbers of the sex chromosomes arise from the father's sperm. Phenotypic consequences have lasting effects on the germ line including infertility, miscarriage, and birth defects. A potential cause of cell signaling defects that result in chromosome abnormalities may be environmental chemicals that impact endocrine system function. Our understanding of how environmental chemicals can disrupt hormone signaling has advanced in recent years and there is emerging evidence that environmental chemicals with hormone disrupting properties can adversely affect spermatogenesis and the occurrence of chromosomal aberrations, possibly through mechanisms of endocrine hormone modulation. Recent epidemiologic data will be presented showing associations between environmental organocholorine exposures (PCBs and p,p'-DDE) and sperm sex chromosome disomy among men who are members of subfertile couples. Among 192 men, we found a significant trend of increasing relative risks for increasing quartiles of p,p'-DDE in XX, XY, and total sex chromosome disomy. Certain subgroups of PCB exposures were associated with a significant increase in the rate of YY, XY, and total sex chromosome disomy for increasing quartiles. These findings will be interpreted within the context of other environmental exposures showing evidence of impacts on the sperm message.

Symposium 7–Application of Epigenetics and Epigenomics Approaches in the Investigation of Human Disease

S31

Coordination of Regulation of DNMT1 Stability. Sedwick WD, Wang Z. Case Western Reserve University Medical School, Cleveland, OH, United States.

DNA Methyl Transferase I, DNMT1, is a critical enzyme for maintenance of both normal and abnormal methylation patterns during DNA replication. Aberrant DNA methylation has been associated with dysfunction of many cellular processes inclusive of cancer, developmental aberration through loss of DNA imprinting, and genomic instability. Our laboratories have recently resolved protein interactions that regulate DNMT1 levels in cells. These studies provide a paradigm for this process that suggests DNMT1 levels are controlled by mediation of its ubiquitination through complex formation with HAUSP (USP7), Tip60, UHRF1, HDAC1 and PCNA. Our evidence supports a model whereby HAUSP and HDAC1 determine DNMT1 stability by maintaining its deubiquitinated state whereas TIP60-mediated acetylation of DNMT1 drives UHRF1- facilitated ubiquitination and subsequent degradation of this enzyme. Results of these studies show that HAUSP inhibition leads to cell death through apoptosis when augmented by HDAC inhibitors. These observations support development of strategies to capitalize on this process through utilization of a combination of HDAC1 and HAUSP inhibitors to inhibit tumor growth.

S32

Abstract not available.

S33

Epigenetic Epidemiology: Examples from Alzheimer's Disease. Rozek LS[1,2], Dolinoy DC[1], Bakulski K[1], Sartor MA[1,2], Hu H[1,2]. [1]University of Michigan, School of Public Health, Ann Arbor, MI, United States, [2]University of Michigan, Medical School, Ann Arbor, MI, United States.

Epigenetic epidemiology can be defined as the study of the effects of heritable epigenetic changes on the occurrence and distribution of diseases. Recent research suggests that epigenetic mechanisms at least partially underlie the contribution of environmental exposures to common diseases. Several lines of evidence indicate that the etiology of late-onset Alzheimer's disease (LOAD) has an environmental etiology that may manifest through epigenetic modifications. In epidemiologic studies of adults, cumulative lifetime lead exposure has been associated with accelerated declines in cognition. In addition, research in animal models suggests a causal association between lead exposure during early life, epigenetics, and LOAD. There are multiple challenges to human epidemiologic research evaluating the relationship between epigenetics, LOAD, and lead exposure. Epidemiologic studies are not well-suited to study a long latency period between exposures during early life and onset of Alzheimer's disease. There is also a lack of validated circulating epigenetics biomarkers and retrospective biomarkers of lead exposure. Members of our research group have shown bone lead as an accurate measurement of historical lead exposure in adults, offering an avenue for future epidemiologic studies. However, this would not address the risk of LOAD attributable to early-life lead exposures. Future studies that use a cohort design to measure both lead exposure and validated epigenetic biomarkers of LOAD will be useful to clarify this important relationship.

S34

Emerging Science and Technologies to Explore Epigenetic Mechanisms: Better Understanding of Human Diseases. Tang WY. Division of Molecular and Translational Toxicology, Department of Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States.

The traditional view that gene and environment interactions control disease susceptibility can now be expanded to include epigenetic reprogramming as a key determinant of human disease development. With the advance of epigenomic technologies, genome-wide maps of histone modification and methylation have been established in a number of different cell types including human embryonic stem cells. Non-coding microRNAs regulating transcription of tens to thousands of genes in mammalian cells has been demonstrated in human diseases, particularly in cancer. Investigation of all epigenetic components allows us to improve our understanding of human diseases. Moreover, epigenetic changes are reversible, offering opportunities for new therapeutic approaches. Determination of epigenetic modifications can provide a new generation of biomarkers for diagnosis, prediction of responses after therapy and prognosis of the diseases. In this presentation, scientific and technological approaches to quantify and identify epigenetic effects in development of human diseases will be examined. Details in experimental design, samples selection, bioinformatics and statistical analysis, and validation systems will be also discussed. Translation of epigenetic study findings into lifestyle recommendations and/or changes in clinical practices can lead to improvement in disease management and, ultimately, public health.

Symposium 8–Site of Contact and Systemic Effects of Formaldehyde Exposure

S35

Evaluation of Formaldehyde As a Potential Cause of Human Leukemia. Goldstein BD. University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA, United States.

Controversial epidemiological findings suggest that formaldehyde exposure is associated with a higher risk of acute myelogenous leukemia (AML) and other hematological cancers. However, it has been difficult on mechanistic grounds to understand how inhalation of this rapidly reactive agent can reach the bone marrow. Also tending to limit acceptance of this epidemiological association was the lack of similarity of non-cancer effects to other known human myeloleukemogens, particularly the absence of pancytopenia in humans or laboratory animals exposed to high formaldehyde levels. However, the recent findings of a pancytopenic effect and chromosomal abnormalities in heavily exposed Chinese workers, if replicated, are indicative of a genotoxic effect of formaldehyde on hematopoietic stem cells that is in keeping with other known human leukemogens. Possible explanations include human susceptibility, particularly as studies in laboratory animals generally fail to show evidence of penetration of formaldehyde into the blood or of blood or bone marrow genotoxicity while studies in humans are suggestive. Another possibility is that myeloid precursors within the nasal mucosa may be the site for leukemogenesis. However, chloromas, which are local collections of myeloid tumor cells, are rarely if ever found in the nose. Understanding a mechanism for human leukemogenesis and mutagenesis by formaldehyde is pertinent to risk assessment for external formaldehyde; for many other chemicals that are metabolized internally to the one carbon pool; and to understand the role of normal formaldehyde formation in the background incidence of human leukemia.

S36

Detection of Formaldehyde-Induced Bone Marrow Toxicity and Allergic Asthma in Experimental Animal Models. Yang X. Cental China Normal University, Wuhan, Hubei, China.

Formaldehyde (FA), a common indoor pollutant in China, is known to cause human cancers. Here we report our recent results from two separated *in vivo* studies on other health effects, including bone marrow toxicity and allergic asthma, induced by inhaled FA. In the bone marrow study, 24 male Wistar rats were exposed to FA at concentrations of 0.0, 0.5, 1.0 and 3.0 mg/m^3 (6 per group) for 72h. DNA-protein crosslinks (DPC) by KCl-SDS assay and DNA strand breaks (DSB) by comet test were measured in the bone marrow cells. FA at low concentrations (0.5 and 1.0 mg/m^3) caused DNA breakage significantly ($p<0.01$); at the higher concentrations (3.0 mg/m^3) increased DPC ($p<0.05$) compared with the control. In the asthma study, 40 Wistar rats divided into five groups were exposed to: saline; ovalbumin (OVA); OVA+0.5 mg/m^3 FA; OVA+3.0 mg/m^3 FA; and 3.0 mg/m^3 FA only. The rats were exposed to FA for 6 h/day for 21 days during OVA immunization or saline treatment. Asthma related outcomes including *in situ* lung function analysis, cytokines, and histological changes in the rat lungs were measured. The airway reactivity, lung histological changes, pulmonary interleukin-4 expression, and eosinophil infiltration in the OVA+FA exposed rats were significantly higher than exposures to either OVA or FA only. Our results suggest that FA exposure induces DNA damage in rat bone marrow and immunotoxicity on respiratory system. Whether or not both toxic effects of FA share a common molecular mechanism (*e.g.* oxidative stress) will be discussed.

S37

Potential Genetic and Epigenetic Effects of Formaldehyde. Swenberg JA, Moeller BC, Herr N, Rager JE, Fry RC, Lu K. University of North Carolina, Chapel Hill, NC, United States.

Formaldehyde is both a highly used industrial chemical and an essential endogenous chemical in every living cell. It is both mutagenic and carcinogenic. Our group has been examining the formation of exogenous and endogenous formaldehyde DNA adducts in site of contact and distant site tissues, as well as evaluating changes in miRNA following exposure of cells to formaldehyde. The DNA adduct studies utilized inhalation exposures of rats and monkeys to [$^{13}CD_2$]-formaldehyde so that adducts arising from exposure could be differentiated from those arising endogenously. The LC-MS/MS method for adducts had the ability to measure as little as 20 attomoles. Endogenous DNA adducts were found in nasal epithelium, lung, liver, spleen, thymus, bone marrow and white blood cells, while [$^{13}CD_2$]-adducts were only present in nasal epithelium. *In vitro* studies of miRNA in formaldehyde-exposed human lung epithelial cells demonstrated down regulation of 89 miRNAs. The DNA adduct data clearly demonstrate that only site of contact tissues have exposure to the genotoxic effects of formaldehyde, supporting the induction of nasal carcinomas. In contrast, the lack of [$^{13}CD_2$]-adducts at distant sites does not support formaldehyde being a leukemogen, as the methods could have seen less than one [$^{13}CD_2$]-adduct per cell in monkey bone marrow, where ~1000 endogenous formaldehyde adducts were present. Additional studies will examine miRNA from rats and monkeys, as well as DNA protein crosslinks. These data will further examine site of contact *vs.* distant site effects of formaldehyde to test the hypothesis that formaldehyde causes leukemia.

S38

Reproductive and Developmental Toxicity of Formaldehyde: A Systematic Review. Duong A[1], Steinmaus C[1,2], McHale CM[1], Vaughan CP[3], Zhang L[1]. [1]School of Public Health, University of California, Berkeley, Berkeley, CA, United States, [2]Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Oakland, CA, United States, [3]Global Health Sciences, University of California, San Francisco, San Francisco, CA, United States.

Formaldehyde, the recently classified carcinogen and ubiquitous environmental contaminant, has long been suspected of causing adverse reproductive and developmental effects, but previous reviews were inconclusive, due in part, to limitations in the design of many of the human population studies. In the current review, we systematically evaluated evidence of an association between formaldehyde exposure and adverse reproductive and developmental effects, in human populations and *in vivo* animal studies, in the peer-reviewed literature. The mostly retrospective human studies provided evidence of an association of maternal exposure with adverse reproductive and developmental effects. Further assessment of this association by meta-analysis revealed an increased risk of spontaneous abortion (1.76, 95% CI 1.20-2.59, p=0.002) and of all adverse pregnancy outcomes combined (1.54, 95% CI 1.27-1.88, p<0.001), in formaldehyde-exposed women, although differential recall, selection bias, or confounding cannot be ruled out. Evaluation of the animal studies including all routes of exposure, doses and dosing regimens studied, suggested positive associations between formaldehyde exposure and reproductive toxicity, mostly in males. Potential mechanisms underlying formaldehyde-induced reproductive and developmental toxicities, including chromosome and DNA damage (genotoxicity), oxidative stress, altered level and/or function of enzymes, hormones and proteins, apoptosis, toxicogenomic and epigenomic effects (such as DNA methylation), were identified. To clarify these associations, well-designed molecular epidemiologic studies, that include quantitative exposure assessment and diminish confounding factors, should examine both reproductive and developmental outcomes associated with exposure in males and females. Together with mechanistic and animal studies, this will allow us to better understand the systemic effect of formaldehyde exposure.

Symposium 9–Whole Genome Mutational Profiling of Environmentally-Induced Cancer

S39

Updates on Next-Generation Sequencing and Bioinformatics. Wheeler DA. Baylor College of Medicine, Houston, TX, United States

Whole genome shotgun sequencing of cancer genomes has afforded an unprecedented view of the mutagenic process that transform normal cells. Now that the basic paradigm has been established in "high quality" tumor samples, in large-scale projects such as TCGA, it is time to focus attention on real world samples from cancer patients. They are often small biopsies that are heavily contaminated with normal cells. This talk will delve into issues of sensitivity for mutation discovery in tumor cells that are a small fraction of the sample from which DNA is purified.

S40

Targeted Resequencing Using the PacBio RS Platform. McPherson JD[1,4], Timms L[1], Brown A[1], Panchel A[1], Zhang T[3], Kamel-Reid S[3,4], Dancey J[1], Siu LL[2,3]. [1]Ontario Institute for Cancer Research, Toronto, ON, Canada, [2]Princess Margaret Hospital, Toronto, ON, Canada, [3]University Health Network, Toronto, ON, Canada, [4]University of Toronto, Toronto, ON, Canada.

Next generation sequencing technology is revolutionizing cancer research with unprecedented amounts of genome-wide data being generated for all tumor types. This is leading to a greater recognition of many of the molecular events associated with the initiation, progression and potential treatment of cancer. The data generated make it abundantly clear that cancer is a complex disease with most tumors being as unique as the individuals they arise in. A personalized medicine approach with the characterization of each tumor to identify key dysregulated pathways will enable more informed prognostic evaluation and more precise targeting of therapies to improve outcomes. The incorporation of this individualized approach in the clinic requires a rapid turn-around from tumor resection or biopsy to informed reporting to the attendant pathologist and clinician. Recently introduced sequencing technologies have revolutionized the field by allowing for rapid targeted resequencing. The Ontario Institute for Cancer Research is piloting the use of one of these instruments, the Pacific Biosciences RS platform and its circular consensus single molecule real time (SMRT) sequencing of PCR amplicons to analyze a validated a set of known oncogenes across tumor samples. The genes in the pilot program have been chosen to provide informative and potentially actionable results as therapeutic targets. This clinical resequencing is being done in collaboration with the University Health Network diagnostic CLIA laboratory to provide validation of all results prior to utilization. Logistics of sample handling, validation of this pipeline and reporting mechanisms are all being evaluated and optimized in a clinical trial setting for eventual routine inclusion into the clinical environment. Funding is provided by the Ontario institute for Cancer Research and the Ministry of Research and Innovation, Ontario.

S41

Paired-End Sequencing Reveals Characteristic Patterns of Structural Variations in Epithelial Cancer Genomes. Bourque G. McGill University & Genome Quebec Innovation Center, Montréal, QC, Canada.

Somatic genome rearrangements are thought to play important roles in cancer development. We optimized a long-span paired-end-tag (PET) sequencing approach using 10-Kb genomic DNA inserts to study human genome structural variations (SVs). The use of a 10-Kb insert size allows the identification of breakpoints within repetitive or homology-containing regions of a few kilobases in size and results in a higher physical coverage compared with small insert libraries with the same sequencing effort. We have applied this approach to comprehensively characterize the SVs of 15 cancer and two noncancer genomes and used a filtering approach to strongly enrich for somatic SVs in the cancer genomes. Our analyses revealed that most inversions, deletions, and insertions are germ-line SVs, whereas tandem duplications, unpaired inversions, interchromosomal translocations, and complex rearrangements are over-represented among somatic rearrangements in cancer genomes. We demonstrate that the quantitative and connective nature of DNA-PET data is precise in delineating the genealogy of complex rearrangement events, we observe signatures that are compatible with breakage-fusion-bridge cycles, and we discover that large duplications are among the initial rearrangements that trigger genome instability for extensive amplification in epithelial cancers.

S42

Landscape of Somatic Alterations in Cancer and Their Relationship to the Environment. Cibulskis K[1], Lawrence M[1], Sivachenko A[1], Sougnez C[1], Nickerson E[1], McKenna A[1], Ramos A[1], Stojanov P[1], Hodis E[1], Zou L[1], Imielinski M[1], Saksena G[1], Drier Y[1], Bass A[1,2], Garraway L[1,2], Meyerson M[1,2], Getz G[1]. [1]The Broad Institute of MIT and Harvard, Cambridge, MA, United States, [2]Dana-Farber Cancer Institute, Boston, MA, United States.

Attempting to understand the genetic basis of cancer can be divided into two goals. First, as complete as possible molecular characterization of individual tumors must be made. The second goal is the interpretation of that data within and across tumor types to elucidate key affected genes and pathways. Next generation sequencing has become a powerful approach in this first goal, not only because of its high-throughput and rapidly falling data generation costs, but also because this single primary data can be for many purposes. Short read data is now commonly used to discover germline variation as well as somatic point mutations, small insertion and deletions, translocations, copy number alterations and RNA expression. We will discuss our experience and approach to the primary analysis of this data type. We will describe some of the critical data QC issues that are essential when handling large amount of next generation sequencing data. We will also show initial results of a pan-cancer analysis including highlighting several cancers such as lung, colon, and skin that are thought to have an environmental component.

S43

The Human SNP Site-Frequency-Spectrum. Gibbs RA. Baylor College of Medicine, Houston. TX, United States.

The characteristics of the spectrum of molecular changes underlying human genetic variation reflects the process of mutation and the subsequent influence of selection and population growth. Among very low frequency variants the influence of selection and population dynamics is less than for common variants. Hence the precise definition and characterization of rare variants in human populations greatly informs, and is informed by, fundamental studies of mutagenic processes and mutation frequencies.

Symposium 10–Transcription and Genetic Instability

S44

Role for Topoisomerase 1 in Transcription-Associated Mutagenesis. Lippert MJ[1], Kim N[2], Jinks-Robertson S[2]. [1]Saint Michael's College, Colchester, VT, United States, [2]Duke University, Durham, NC, United States.

Transcription influences genomic instability in complex ways. High levels of transcription stimulate spontaneous mutagenesis and recombination, known as transcription-associated mutation (TAM) and recombination (TAR), respectively. In addition, bulky transcription-blocking lesions trigger transcription coupled repair (TCR), a subpathway of nucleotide excision repair. The transcription machinery may also act as a DNA damage sensor and participate in the cellular stress response. The mechanisms of these various processes are becoming clearer. Recently, we identified a novel mechanism of TAM using yeast as a model system. High levels of transcription stimulated CAN1 forward mutation rate 12.3 fold (from 10.7- to 132 x 10^{-8}) and shifted the mutation spectrum dramatically. Specifically 2-5 bp deletions comprised 55% of the high-transcription spectrum compared to 2.4% of the low-transcription spectrum. Moreover the short deletions occurred predominantly at hotspots characterized by short repeats. In contrast to other classes of transcription-associated mutations, rates of short deletions were unaffected in homologous recombination-deficient or DNA polymerase ζ deficient strain backgrounds. However, the transcription-associated deletions were completely dependent upon Topoisomerase 1, the enzyme which addresses transcription-associated supercoiling in yeast. In a normal reaction Topoisomerase 1 relaxes supercoiled DNA by cutting one strand, attaching covalently to the 3′-DNA end, relieving a supercoil by rotation of the free 5′-DNA end, and resealing the DNA backbone. We propose that high levels of transcription generate irreversible Top1 cleavage complexes that, when present within a repeat sequence, create the mutation intermediate leading to short deletions.

S45

Transcription Impacts Genomic Stability *via* Multiple Mechanisms. Kim N[1], Lippert M[2], Jinks-Robertson S[1]. [1]Duke University Medical Center, Durham, NC, United States, [2]St. Michael's College, Colchester, VT, United States.

In *Saccharomyces cerevisiae*, the level of transcription is associated with the level of genome instability in the region. Highly activated transcription increases both the rate of mutagenesis (transcription-associated mutagenesis or TAM) and the rate of recombination (transcription-associated recombination or TAR). Increased single-strandedness, accumulation of DNA damage, replication/transcription collision, extensive RNA-DNA hybrid, and increased torsional stress are some of the possible factors connecting the highly activated transcription and genome instability. Using a mutation reporter system where the transcription is regulated from a pTET promoter, we identified a unique mutation signature of TAM in base excision repair (BER) deficient background. When transcription is activated, uracil accumulates in DNA. Then, abasic sites generated by the uracil DNA glycosylase lead to small frameshifts and/or base changes mediated by translesion synthesis mechanism. We also observed a dramatic increase in the 2 to 5 nt deletion mutations when CAN1 gene was transcribed from highly activated pGAL1 promoter. The deletions occurred at discrete hotspots composed of small tandem repeats and were dependent on the catalytic function of the yeast type IB topoisomerase Top1. Disruption of RNase H2 function increased the rate of a subset of these deletion mutations suggesting that ribonucleotide incorporation in DNA is responsible for these unique mutations. The data presented here argue that uracil and ribonucleotide are highly mutagenic DNA lesions specific to actively transcribed regions.

S46

Transcription-Associated Recombination at Replication Forks in Mammalian Cells. Helleday T[1,2]. [1]Department of Genetics, Microbiology, and Toxicology, Stockholm University, Stockholm, Sweden, [2]Gray Institute for Radiation Oncology & Biology, University of Oxford, Oxford, United Kingdom.

Homologous recombination (HR) is plays a major role in repairing replication-associated lesions. However, the nature of the sporadic lesions triggering HR at replication forks is poorly understood, especially in mammalian cells. Transcription is another cellular process, which has emerged to have a connection with HR. Transcription enhances HR, which is a ubiquitous phenomenon referred to as transcription-associated recombination (TAR). Here, I will present mechanisms and the underlying genetic requirements for TAR in mammalian cells, which demonstrate that this is a distinct pathway for HR. Not only is TAR important to maintain genomic integrity, it may also cause genetic instability. Hence, TAR is a double-edged sword and plays a role in both preventing and inducing genetic instability. Here, I will present emerging data from our laboratory that link transcription to oncogene-induced replication stress in early cancer development.

S47

Transcription-Blockage by Lesions and Unusual DNA Structures. Hanawalt PC, Salinas-Rios V, Belotserkovskii BP. Stanford University, Stanford, CA, United States.

Lesions in the transcribed DNA strand can be sensitively detected by blocked translocating RNA polymerases, to initiate transcription-coupled excision repair (TCR). DNA sequences that can form non-canonical DNA structures may also block transcription and these have been implicated in genomic instability (*e.g.* G-4 quadruplexes, H-DNA, Z-DNA and slip-outs generated in triplet repeat sequences). G-rich homopurine-homopyrimidine tracts can also block transcription in an orientation-, length-, and supercoiling-dependent manner (Belotserkovskii *et al.* 2010). Unusual DNA structures might trigger a gratuitous form of TCR, which could be mutagenic and/or might enhance transcription blockage by some types of DNA lesions (for review see: Hanawalt and Spivak, 2008). Transcription has been implicated in triplet repeat instability and there is genetic evidence that this is mediated by TCR (Y.Lin *et al.*, 2007,2009). The proposed mechanism involves futile cycles of TCR at sites of transcription arrest where slip-outs have formed hairpins. We have shown that all structures formed by CAG and CTG repeat slip-outs can cause transcription arrest *in vitro*. Slip-outs of $(CAG)_{20}$ or $(CTG)_{20}$ repeats on either DNA strand arrest RNA polymerase II or T7 RNA polymerase in HeLa cell extracts, although T7 RNA polymerase in a purified system is not affected by slip-outs. Our findings are consistent with a transcription-dependent repeat instability model in which gratuitous TCR might lead to either expansion or contraction of the repeat copy number (Salinas-Rios *et al.* 2011). The model is relevant to the etiology of hereditary neurological syndromes, such as Huntington's disease, characterized by triplet nucleotide repeat expansion.

S48

The Transcriptome and the DNA Repairome. Ljungman M, Prasad J, Veloso A, Wilson TE, Paulsen MT. University of Michigan, Ann Arbor, MI, United States.

It is well known that DNA damage affects transcription and transcription affects DNA repair. To comprehensively study transcription on a genome-wide basis we are developing BruChase-Seq and BrUV-Seq which are approaches based on bromouridine (BrU) pulse-chase labeling followed by deep sequencing. We are currently using BruChase-Seq and BrUV-Seq to assess the effects of environmental genotoxic agents on i) the synthesis and stability of all mRNAs and non-coding RNAs, ii) patterns and kinetics of splicing, iii) mapping of transcription start sites and poly(A) sites and iv) mapping of enhancers and other transcribed intergenic elements. In BrUV-Seq, UV light is used to introduce random transcription-blocking lesions in the genome prior to the bromouridine-labeling procedure. The results show that UV-irradiation impairs elongation but not initiation of transcription resulting in a marked enrichment of the density of the deep sequencing hits toward the 5'-end of genes making BrUV-Seq very useful in mapping transcription start sites. We are also developing approaches to study genome-wide induction and repair of DNA damage by coupling established DNA damage detection techniques with next generation sequencing. By comparing the "transcriptome" with the "DNA repairome" we hope to gain novel genome-wide insights into how transcription and DNA repair rely on and influence each other.

Symposium 11–Low-Dose Mutagenesis and Carcinogenesis

S49

Low-Dose Mutagenesis and Carcinogenesis: Why Is This Issue Important? Zeiger E. Errol Zeiger Consulting, Chapel Hill, NC, United States.

Mutagenicity and carcinogenicity testing are typically performed at high doses up to toxic levels. In contrast, human exposure to chemicals of concern, other than many prescription pharmaceuticals, is at doses that may be orders of magnitude below those used in the tests. There is an assumption that mutagenic responses are linear at low doses and therefore, when an agency conducts a cancer risk assessment for a mutagenic substance, the high test dose responses are extrapolated down to zero dose using a low-dose linear relationship as the default model. Such a low-dose linear extrapolation typically leads to more conservative risk calculations than if the response was sublinear or exhibited a threshold. A number of studies on mutagenic carcinogens have recently appeared that have examined the low-dose mutagenic response to assess whether the linear default model accurately reflects the actual response, and the biology leading to the response. Some of the research and assumptions that do not support the low-dose linear assumption will be presented.

S50

Low-Dose Mutagenicity Case Studies: MMS and MNU. Pottenger LH. The Dow Chemical Company, Midland, MI, United States.

The historical view of genotoxic responses as "linear, no-threshold" has not encouraged evaluation of the low-dose region of mutation dose-response relationships. Over the past 15^{+} years, this historical view has been re-evaluated, leading to collection of mutation data designed to determine no-observed-genotoxic-effect-levels (NOGELs). Identification of clear NOGELs for genotoxicity provides extremely useful information to the risk assessment process, in particular informing the risk assessor on the probability of an adverse event at low, environmentally relevant exposure levels, of key importance in assessing human risk. In fact, a growing body-of-evidence supports the existence of NOGELs or response thresholds for a number of directly DNA-reactive mutagenic agents, both *in vitro* and *in vivo*, as opposed to a linear, no-threshold dose-response. While the simple observation of a "hockey-stick" dose-response curve is not sufficient to establish a threshold for a mutagenic response, it adds significantly to the weight-of-evidence. Ideally, collection of robust empirical dose-response data will be supported with an analysis of biological plausibility for the observed threshold. Both methyl methanesulfonate (MMS) and N-nitroso-N-methylurea (MNU), as directly DNA-reactive chemicals and typical positive control chemicals for mutation assays, constitute good examples to evaluate the shape of the low-dose dose-response for mutations. Based on mostly recent published data, it is clear that non-linear/threshold dose-responses can be demonstrated for MMS and MNU, both for induction of gene mutations and for chromosomal events. Statistical methods for evaluation of these data have evolved and demonstrate improved robustness to differentiate between linear and non-linear dose-response models.

S51

Low-Dose *In Vivo* Study of Acrylamide. Zeiger E. Errol Zeiger Consulting, Chapel Hill, NC, United States.

Acrylamide is an industrial chemical used in polymer manufacture and is formed in foods processed at high temperatures. It induces chromosome aberrations and micronuclei (MN) in somatic cells of mice and gene mutations in transgenic mice. This study evaluated the low-dose MN response in mouse bone marrow and the shape of the dose-response curve. Mice were treated orally with acrylamide for 28 days with doses from 0.125 to 24 mg/kg/day, and MN were assessed in peripheral blood reticulocytes (RETs) and erythrocytes by flow cytometry. Liver glycidamide DNA adducts and acrylamide and glycidamide hemoglobin adducts were also determined. Acrylamide produced a weak, statistically significant MN response at 6.0 mg/kg/day, or greater, in RETs and at 4.0 mg/kg/day or greater in normochromatic erythrocytes (NCEs). The MN responses at the lower doses were indistinguishable from the concurrent and historical controls. The adducts increased linearly at a much greater rate than the MN. When the MN-NCE values were compared to administered dose, the response was marginally more consistent with a linear model. When hemoglobin or DNA adducts were used as the dose metric, the response was significantly nonlinear, and models that assumed a threshold dose of 1 or 2 mg/kg/day provided a statistically significant better fit than a linear model. The MN-RET dose-response had greater variability than the MN-NCE response and was consistent with linearity and with a threshold at 1 or 2 mg/kg/day, regardless of the dose metric. These data support a threshold for acrylamide in the bone marrow MN test.

S52

Low-Dose Carcinogenicity Studies. DeMarini DM. U.S. Environmental Protection Agency, Research Triangle Park, NC, United States.

One of the major deficiencies of cancer risk assessments is the lack of low-dose carcinogenicity data. Most assessments require extrapolation from high to low doses, which is subject to various uncertainties. Only 4 low-dose carcinogenicity studies and 5 low-dose biomarker/pre-neoplastic studies have been performed. The 4 carcinogenicity studies involved exposures of 24,192 mice to 2 acetylaminofluorene, 4,080 rats to nitrosamines (NDMA and NDEA), 40,000 rainbow trout to dibenz[a,l]pyrene, and 20,00 trout to aflatoxin B1. The low-dose biomarker/pre-neoplastic studies involved exposure of 1,145 rats to MeIQx, 2,000 rats each to DEN or DMN, 1,920 rats to PhIP, and 50 rats to potassium bromate. In most cases there was some evidence for a threshold effect for the induction of cancer or biomarkers of cancer. However, absolute proof of a threshold effect for carcinogenicty could not be demonstrated unambiguously by any of the carcinogenicity studies due to the limited number of animals used. The induction of stable DNA adducts was not predictive of tumors in the mouse and trout studies. All of the biomarkers evaluated, including stable DNA adducts, oxidative damage, mutation, and pre-neoplastic foci, exhibited threshold effects, *i.e.*, they were not inducible/detectable at the lowest doses tested. The limited data available indicate that genotoxic carcinogens can exhibit threshold effects for the induction of carcinogenic biomarkers and cancer. Carcinogenicity studies of a size sufficient to detect 1 cancer per million animals directly, without extrapolation, are currently not possible to perform. [Abstract does not necessarily reflect the views or policies of the U.S. EPA.]

S53

Analysis of Low-Dose Mutagenic Responses and the Applicability of Genotoxicity Tests for Carcinogen Potency Prediction. Hernandez LG[1], Johnson GE[2], Pottenger LH[3], van Benthem J[1]. [1]Health Protection Research, National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands, [2]DNA Damage Group, School of Medicine, Swansea University, Swansea, United Kingdom, [3]Toxicology and Environmental Research and Consulting (TERC), The Dow Chemical Company, Midland, MI, United States.

Genotoxicity tests have generally been thought of in a yes/no fashion for hazard identification but understanding dose-response relationships is becoming increasingly important for two main reasons. Firstly, to address whether genotoxic carcinogens have thresholds or 'safe exposure doses'; particularly given that ethyl methane sulphonate (EMS), a DNA-reactive compound, now has a well established threshold dose-response. Secondly, to assess whether parameters derived from *in vivo* genotoxicity studies can be used to predict the carcinogenic potency of chemicals. The *in vitro* genetic toxicity (IVGT) quantitative subgroup of the ILSI/HESI has created an excellent database of *in vitro* and *in vivo* genotoxicity data currently encompassing EMS, methyl methanesulphonate, methyl nitrosourea and ethyl nitrosourea. In order to assess which methods are suited for the determination of 'safe exposure doses', the benchmark dose (BMD) approach was applied to *in vivo* and *in vitro* micronucleus data and compared to other statistical approaches such as the hockey stick, the bilinear approach, and the no-observed genotoxic effect level (NOGEL). Preliminary comparisons demonstrate that the lowest confidence interval of the BMD ($BMDL_{10}$) was generally lower than the threshold doses and NOGELs derived by the other statistical methods, and lower than the $BMDL_{10}$ from carcinogenicity studies. In an independent effort, work at the RIVM has evaluated potency correlations between the lowest $BMDL_{10}$ for *in vivo* genotoxicity (micronucleus and transgenic rodent mutation assay) and carcinogenicity in 18 compounds (potency range ~9000). Preliminary data demonstrate the applicability of different statistical approaches to assess genotoxicity data and possibly for developing carcinogen potency predictions.

S54

Dose-Response Issues in the Regulation of Chemicals. Schoeny RS. U.S. EPA Office of Water, Washington DC, United States.

U.S. EPA and other regulatory groups often define "science policies" as a means of moving forward with risk assessments and management decisions in the absence of all the data these bodies would prefer to have. These science policies may include the use of default assumptions, values and methodologies. Parameters for development and use of science policies have been published in Agency Guidelines as well as in reports by the U.S. National Academies of Sciences such as Science and Judgment and the more recent Science and Decisions (the Silver Book). The U.S. EPA 2005 Cancer Guidelines stated a preference for use of data over defaults and mode of action (MOA) as a basis for key risk judgments; regulatory groups in Europe and elsewhere express similar preference. Furthermore the Cancer Guidelines gives the highest priority to use of biologically based dose-response models (BBDR), when supported by data, over use of any default. A BBDR is preferred to default, linear, low-dose extrapolation from a point of departure for carcinogens thought to act through a mutagenic MOA. It is noted that risk assessment is only one input to the process whereby choices among risk management options are made. Policy (other than science policy and in some instances defined in statute) is a major factor in this decision process. Examples of use of policy based on science *vs.* science policy will be presented. The opinions in this abstract are those of the author and may not reflect policies of U.S. EPA.

Symposium 12–Risks Associated with Inadvertent Exposures to Pharmaceutical and Prescription Drugs

S55

Inadvertent Exposures to Pharmaceutical Drugs: Overview. Weston A. NIOSH, Morgantown, WV, United States.

Inadvertent exposure to pharmaceutical drugs can occur through multiple routes (drug development and manufacturing worker exposure/pharmacist exposure/healthcare worker exposure/patient exposure), several of which will be explored during this symposium. The first presentation will address research/development and manufacturing worker exposures, where risk assessment may be based on chemical structure, and comparisons made with known, structurally similar drugs. Also relevant to this is the use of equipment in the context of synthesis of a previous drug, where residual materials may contaminate a subsequent preparation. Therefore Risk-based Manufacture of Pharmaceutical Products is an important consideration. The second presentation will consider patient safety in the context of such residual contamination of pharmaceutical products. Specifically, the acceptable level for genotoxic impurities occurring during drug synthesis, the threshold for toxicological concern, and acceptable daily exposure will be explored. the third presentation will consider exposures to healthcare workers, especially in the context of antineoplastic drugs. The presence of antineoplastic drugs and their metabolites in the urine of nurses and other healthcare professionals confirms exposure. in each of these three exposure scenarios, pharmaceutical agents can cause genotoxic and/or mutagenic damage. Studies of workers and patients inadvertently exposed to pharmaceutical agents have included testing of surrogate tissues (peripheral white blood cells and buccal cells) for DNA damage; *in vitro* mutagenicity testing of urine has also been used as a marker of exposure. These and other testing measures have been employed to assess the efficacy of engineering controls and personal protective equipment to prevent inadvertent exposures.

S56

Risk-Based Manufacture of Pharmaceutical Products. Mahadevan B. Abbott Laboratories, Abbott Park, IL, United States.

The global pharmaceutical industry and regulators are responding to the challenge of significantly improving the way drug development and manufacturing is managed. In this regard, the use of the health-based limits as the basis for risk assessment in the manufacture of pharmaceutical products is a scientifically sound approach. Risk-MaPP (Risk-based Manufacture of Pharmaceutical Products), an ISPE (International Society of Pharmaceutical Engineering) guidance document provides good practices that can help a company develop an approach that is effective, cost-efficient, and in compliance with existing regulations and related guidance. In this presentation, the concept of Risk-MaPP will be elaborated with particular emphasis on deriving an Acceptable Daily Exposure (ADE) for pharmaceuticals. The application of the Threshold of Toxicological Concern (TTC) concept to pharmaceutical manufacturing operations will also be illuminated with examples.

S57

Considerations for Risk Assessments of Genotoxic or Carcinogenic Impurities in Industry. Nicolette J. Abbott Laboratories, Abbott Park, IL, United States.

Pharmaceuticals are tested for their potential to cause genetic damage prior to being tested for safety and/or efficacy in clinical trials. Drugs that test positive in *in vitro* genetox studies are vetted in multiple *in vivo* and mechanistic studies prior to administration to volunteers in first in human trials. To make a synthetic compound, reactive starting materials and intermediates used in the route. Solvents and reagents can combine with these or together creating unwanted impurities in the final active pharmaceutical ingredient (API). These impurities themselves could have genotoxic potential. Historically, impurities in the API did not have to be identified unless they exceeded 0.1%, and qualification wasn't required unless above 0.15% in the API. In addition, these guidelines applied to the final marketed drug, with no guidance for drugs in clinical development. Unlike other toxicities, mutagenicity has been considered a non-thresholded phenomenon. To protect clinical trial subjects, recent guidance from the European Medicines Agency (EMEA) suggested that a 1.5 microgram per day or lower intake of genotoxic carcinogens would result in less than 1 additional cancer per 100,000 and that this is an acceptable level for genotoxic impurities found in a synthetic route. This is known as the threshold for toxicological concern or TTC for pharmaceuticals. Additional refinement has been made to account for short duration exposures in clinical trials without addition risk to the subject. This presentation will discuss evaluation of synthetic routes with emphasis application of the TTC as well as the staged-approach along with examples.

S58

Preventing Occupational Exposures to Antineoplastic Drugs in Health Care Settings. Connor TH. National Institute for Occupational Safety and Health, Cincinnati, OH, United States.

Introduction: Occupational exposure to antineoplastic drugs has been a concern of healthcare professionals since the 1970s following reports of secondary cancers in patients treated with these drugs. The detection of mutagens in the urine of nurses first alerted healthcare professionals to the potential for exposure to antineoplastic drugs in the workplace. Since many of the anti-cancer drugs cause DNA damage, properly designed tests for genotoxicity can reflect exposure to these drugs. Methods: Peer-reviewed articles dealing with biomarkers of genotoxic damage associated with occupational exposure to antineoplastic drugs published between 1990 and 2011 were reviewed. The studies employed various types of assays for biomarkers of genotoxicity. In addition to urine mutagenicity, biomarkers included chromosomal aberrations, sister chromatid exchanges, induction of micronuclei and DNA damage, plus several miscellaneous endpoints. Results: During this period, 99 studies were identified that evaluated differences in biomarkers of genotoxic damage in healthcare workers as compared to matched control groups. The majority of the studies was cross-sectional in design and often had the limitation of small sample size. Some studies evaluated interventions aimed at reducing exposure to these drugs. Approximately two-thirds of the studies reported an association between occupational exposure to antineoplastic drugs and the biomarker of genotoxicity. In most studies, interventions reduced the frequency of genotoxic damage. Discussion: Although the long-term consequences of the exposure to antineoplastic drugs are not fully understood, given their genotoxic potential, efforts should be undertaken to reduce potential exposure to these drugs in the workplace.

Symposium 13–Using DNA Adducts in Risk Assessment: Approaches, Considerations, and Significance

S59

ILSI/HESI DNA Adducts Project Committee: Review of Case Study Outcomes: Tamoxifen, AFB_1, and VCl. Pottenger LH. The Dow Chemical Company, Midland, MI, United States.

The biological significance and role of DNA adduct data in risk assessment are debated. An ILSI/HESI Committee published a systematic approach for the evaluation of DNA adduct data in a key event dose-response framework for a mutagenic mode-of-action (MOA) analysis for cancer risk. The approach stresses the need to create a context for adduct data in conjunction with other key types of data such as dosimetry, mechanistic response data, and tumor incidence. This systematic approach was applied to data for three chemicals to illustrate its use: aflatoxin B_1, tamoxifen, and vinyl chloride. These chemicals presented a variety of characteristics and some specific challenges for adduct data interpretation, such as presence of background/endogenous adducts, different MOAs for rodents *vs.* humans, and data quality and reliability. Analysis of these case studies led to a set of general principles for evaluating the role of DNA adduct data in the MOA, including the following: Target tissue and adduct type depend on exposure concentration, duration, and internal dose determinants such as physico-chemical properties, anatomical and physiological factors, and ADME processes. Adduct profiles can change with duration or dose, due to differences in repair/persistence of specific adducts. Both characterization and structural identification are necessary for DNA adduct use in MOA assessment. Key conclusions include the following: DNA adduct data cannot be used in isolation to determine a mutagenic MOA; DNA adducts represent biomarkers of exposure and not of effect; and DNA adduct data alone are informative but not sufficient to assign a mutagenic MOA.

S60

Application of a Decsion Analytic Approach to Case Studies. Jarabek AM. U.S. EPA, National Center for Environmental Assessment, Research Triangle Park, NC, United States.

Recent assessment approaches use weight of evidence and human relevance frameworks to evaluate evidence on the mode of action (MOA) for a chemical. To date, what is often lacking is an explicit expression of data quality, utility, and reliability to support claims of causality for a given key event(s). This expression is important because judgments concerning data on parameters for specific steps influence the confidence in the ultimate decision regarding causality. A two-step decision analytic approach to evaluate causality is proposed. The 1st step is to populate a conceptual MOA model with specific data on parameters. Description of the pathogenesis process is divided into characterization of two key components: dosimetry (toxicokinetics) and response (toxicodynamics). The 2nd step entails evaluating both the ability of the data to describe or represent the particular parameter or process, and the extrapolation premises or assumptions required to apply these data to the human disease target context. This requires explicit evaluation of the data from various observational contexts: *in vitro*, *in vivo* laboratory animal and human studies. Data are assigned to evidence categories (direct empirical, semi-empirical, empirical correlation or theory-based inference) to assess relevance. Coherence of data within an observational context and then across contexts is used to arrive at a summary judgment of causality for each of the two characterizations. These are combined to arrive at overall confidence in the conclusion regarding the causal role of the proposed key event(s). (These views are those of the author and do not represent U.S. EPA policy).

S61

Mutagenesis and Repair of O^6- and N7-Alkylguanine DNA Adducts. Fuchs RP[1], Mazon G[1], Modesti M[1], Phillipin G[1], Gasparutto D[2], Cadet J[2]. [1]CNRS, UPR 3081, Marseille, France, [2]CEA, LAN, Grenoble, France.

The mutagenicity of O^6- and N7-alkylguanine adducts formed by ethylene oxide (EO) and propylene oxide (PO) was investigated by the use of single-adducted plasmids. N7-alkylguanine adducts were found to be intrinsically non-mutagenic provided the apurinic sites that may form spontaneously are removed prior to introduction of the plasmids into cells. In contrast, O^6-alkylG adducts are highly mutagenic due to their capacity to efficiently mispair with T during replication, triggering G->A transitions. For these lesions, mutagenesis is largely prevented by repair strategies *via* alkyltransferases (AT) or Nucleotide Excision Repair (NER) pathways. Recently, we have shown that the alkyltransferase-like gene ybaZ (eATL) enhances repair of these adducts by Nucleotide Excision Repair. In addition, methyl-directed Mismatch Repair (MMR) is known to trigger sensitivity to methylating agents *via* a mechanism that involves recognition by MutS of the O^6-mG:T replication intermediates. We show that eATL prevents MMR-mediated attack of the O^6-alkylG:T replication intermediate for the larger alkyl groups but not for methyl. *In vivo* data are compatible with the occurrence of repeated cycles of MMR attack of the O^6-alkylG:T intermediate. *In vitro*, the eATL protein efficiently prevents binding of MutS to the O^6-alkylG:T mispairs formed by the larger alkyl groups but not by methyl. In conclusion, eATL not only enhances the efficiency of repair of these larger, O^6-alkylG adducts by NER, but it also shields these adducts from MMR-mediated toxicity. This is the first report demonstrating the lack of intrinsic mutagenic effect by N7-alkylG adducts induced by EO or PO.

S62

Relevance of DNA Adduct Approaches to Regulatory Risk Assessment. Schoeny RS. U.S. EPA Office of Water, Washington, DC, United States.

Genetic toxicology data are a major input to risk assessments: to screen chemicals for mutagenicity or other types of genetic damage related to carcinogenic potential; as part of a weight of evidence judgment that a chemical causes cancer or other effects; in determination of a mode of action (MOA) for an adverse effect. U.S. EPA in the 2005 Cancer Guidelines stated a preference for use of data over defaults and MOA as a basis for key risk judgments; regulatory groups in Europe and elsewhere express similar preference. Regulatory risk assessors are increasingly aware that their work is most appropriately not an academic exercise, but rather targeted to provide information needed to discriminate among risk management options. This is characterized as "utility of risk assessment" or fit for purpose. EPA is developing a framework focused on problem formulation to ensure that risk assessments provide the most useful information for risk management options. Consideration of DNA adduct data in the context of cancer MOA provides some examples. Understanding the extent to which exogenous exposure contributes to adducts resulting from normal metabolism informs the dose-response assessment, in turn leading to choices (*e.g.*, acceptable exposure levels). Identification of chemical-unique promutagenic adducts (aflatoxin) not only suggests useful exposure biomarkers, but also potential key events in a MOA. By contrast, knowledge that a chemical's human MOA is unrelated to its adducts (tamoxifen) is just as useful. The opinions in this abstract are those of the author and may not reflect policies of U.S. EPA.

Symposium 14–Environmental Oil Contamination: Effects on Marine Life and Human Life

S63

Voyage 2 of the Odyssey: The Impact of the Oil Crisis on Health Using Whales As an Indicator Species. Wise, Sr. JP[1,2], Kerr I[2], Wise, Jr. JP[1,2], Wise CF[1,2], Wise SS[1,2], Wise J[1,2], Gianios, Jr. C[1,2], Wallace B[2], Glass I[2], Walker R[2], Huang S[1,2], LaCerte C[1,2], Holmes A[1,2]. [1]Wise Laboratory of Environmental and Genetic Toxicology, Portland, ME, United States, [2]Ocean Alliance, Lincoln, MA, United States.

The 2010 Gulf of Mexico oil crisis began 50 miles offshore in more than 5,000 feet of water. It released approximately 200 million gallons of oil into the Gulf and was the worst marine oil spill in U.S. history and one of the worst in world history. At least 2 million gallons of chemical dispersants were administered to the oil. This approach was the first use of these chemicals on such a massive scale. The toxicity of the dispersants and the dispersed oil is poorly understood. It is still unclear, from a scientific point of view, whether application of dispersants was successful or not. States like Maine and others with large oil-related activity face significant risks of large oil spills. Thus, it is essential to learn the necessary lessons from this crisis. To address this need, we are conducting The Gulf of Mexico Offshore Toxicology Study. This multiyear study uses this crisis as a case study of oil pollution and seeks to understand the impacts of chemical dispersants, crude oil, dispersed oil and oil-related metals on marine life and human health. It focuses on whales as representative species because they integrate all routes of exposure, serve as sentinels for human health, and are important in the ocean ecosystem. It studies the accumulation of these agents in whales and their environment and considers their impact on DNA in cultured whale and human cells. When complete, it will provide insight into the toxic effects of these agents in offshore waters.

S64

NIOSH Health Hazard Evaluation Conducted in the Aftermath of the Deepwater Horizon (DWH) Disaster. Weston A. NIOSH, Morgantown, WV, United States.

Following the DWH disaster, BP requested that NIOSH evaluate exposures and health effects among workers involved in response activities. NIOSH began in early May by developing a roster of more than 50,000 potential workers. Then NIOSH activities were focused on exposure and health assessment at specific worksites. These included on-shore evaluations (shore clean-up, wildlife rehabilitation, equipment decontamination, and waste stream management) and off-shore evaluations (source control, in-situ burns, and booming/skimming/dispersant operations). Nine interim reports (http://www.cdc.gov/niosh/topics/oilspillresponse/gulfspillhhe.html) have been released, including: 1) industrial hygiene surveys, symptom surveys, and medical interviews during small-area oil dispersant missions on two vessels; 2) infirmary logs for response workers: gastrointestinal, dermatological, respiratory, ophthalmic, and dental symptoms; injuries, bites and stings; heat-related disorders; and other conditions; 3) industrial hygiene activities aboard a vessel during an oil skimming mission; 4) measurement of airborne concentrations of benzene, 2-butoxyethanol, and CO on several vessels dispersing foam patches on the water; 5) exposure assessment, site characterization, and symptoms assessment among wildlife cleaning workers; 6) comparison symptoms among off-shore workers to those among shore workers not having oil, dispersant, or cleaning material exposures; 7) exposure assessment and symptom questionnaire at 67 shore-cleaning worksites; 8) health hazards at equipment and boat repair/decontamination and waste management worksites; 9) laboratory analysis of various bulk samples: fresh and weathered oil and burnt oil residue, dispersant foam, and drilling mud. Principal NIOSH recommendations related to worker training, use of personal protective equipment, heat stress management, and proactive reporting of illness/injury.

S65

Are Seafood Safety Considerations Adequate to Prevent Chronic Health Effects of the Deep Water Horizon Oil Spill on Coastal Louisiana Residents? Williams PM. University of New Orleans, New Orleans, LA, United States.

In an effort to determine the safety of seafood following the BP Deepwater Horizon Oil Spill in the Gulf of Mexico on April 20, 2010, a protocol for testing of twelve PAHs was developed by the U.S. Food and Drug Administration. Numerous deficiencies and inaccuracies compromise the quality of this seafood safety program. More than a hundred Polycyclic Aromatic Hydrocarbons (PAHs) are found in crude oil and are of particular toxicological concern due to synergism among the complex mixtures. The FDA Center for Food Safety and Applied Nutrition/Dauphin Island, reported that as of February 23, 2011 a total of 1,406 seafood specimens had been tested. Approximately 281 specimens are tested per month. In comparison 1.3 billion pounds of commercial fish and shellfish were harvested from the five U.S. Gulf states in 2008. Recovery of PAHs from spiked control samples for Gulf seafood analysis were reported to be 40 to 120 % of controls. The risk assessment formula for Gulf seafood safety testing specifically ignores the synergism of the PAH mixture. Reproductive and developmental toxicity are excluded from consideration in the seafood safety protocol. Daily consumption rates used in the risk assessment calculations are not appropriate for the high seafood consumption of coastal Louisiana residents. Specimens actually tested from pre-market populations cannot adequately represent the voluminous harvest of fish and shellfish from the Gulf of Mexico. Current extraction procedures should be further investigated given the inaccuracy of recovery of PAHs from control samples.

S66

Overview of the Toxicity of the Oil Dispersant Corexit. DeMarini DM. U.S. Environmental Protection Agency, Research Triangle Park, NC, United States.

The anionic surfactant dioctyl sodium sulfosuccinate (DOSS) is in the oil dispersant Corexit 9500A, which was used in the Deepwater Horizon Oil Spill. Analysis of water from the area of Deepwater Horizon showed that DOSS biodegraded little or at a slow rate several months after application of Corexit. Corexit did not alter the biodegradation rate of oil in lab studies. Corexit is not mutagenic to *Salmonella* and does not cause mutagenic urine in rats exposed orally to it or oil + Corexit. Corexit was not an endocrine disruptor *in vitro*. Corexit results in greater exposure of marine organisms to hydrocarbons (PAHs) from oil in waters where the salinity is low, such as in coastal waters. Although Corexit or Corexit + oil are generally more toxic to early-life stages than to adults, species differences complicate this generalization. Corexit is less toxic than oil alone, and the toxicity of oil or oil + Corexit to aquatic organisms is similar when based on measured water-column values and the concentration of non-volatile hydrocarbons. The concentrations of dissolved hydrocarbons in the water column are similar for oil or Corexit + oil, and most toxicity appears to be due to the concentration of the dissolved (soluble) hydrocarbons. Corexit also puts some portion of the hydrocarbons into colloidal suspension, and these are less associated with toxicity than the dissolved (soluble) hydrocarbons. [Abstract does not necessarily reflect the views or policies of the U.S. EPA.]

Symposium 15–Intestinal Microbiota: A Key Player in Obesity, Genomic Instability, and Lymphoma

S67

Intestinal Microbiota Affect Genetic Instability, Longevity and Lymphoma Latency in Atm$^{-/-}$ Mice. Schiestl R[1], Yamamoto M[1], Presley L[2], Borneman J[2], Reliene R[1], Westbrook A[1], Chang C[1]. [1]UCLA, Los Angeles, United States, [2]UCR, Riverside, United States.

Ataxia Telangiectasia (AT) is an autosomal recessive disorder characterized by motor dysfunction, chromosomal instability, radiosensitivity, oxidatively stressed phenotype and high incidence of cancer. ATM is a central protein in DNA repair, checkpoint control, and redox balance. Significant unexplained inter- and intra-laboratory differences in the lifespan of isogenic Atm-deficient mice from 2 months to 11.5 months exist in the literature. We found that by experimentally changing the intestinal microbiota, which plays a critical role in our health, we can affect genetic instability, longevity and lymphoma latency in Atm-deficient mice. We show that isogenic Atm$^{-/-}$ mice with different intestinal microbiota have significant variations in DNA deletions between 10 and 43%, have a significant variation of median lifespan between 32 and 51 weeks, and the median lymphoma latency varied significantly between 25 and 60 weeks. Our findings present an uncharacterized experimental variable, which may have tremendous implications: first in the prevention or delay of lymphoma in AT patients, and secondly for basic research, in which inter- and intra-laboratory differences in Atm-deficient and possibly other cancer predisposed mouse models are in part or entirely explained by differences in intestinal microbiota.

S68

The Intestinal Host-Microbial Ecosystem: A Systems Biology Approach to Inflammation and Cancer Risk. Braun J[1], Li X[1], Tong M[1], LeBlanc J[1], Goodglick LA[1], Borneman J[2]. [1]Department of Pathology and Lab Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA, United States, [2]Department of Microbiology, UC Riverside, Riverside, CA, United States.

Aberrant interactions between the host and the intestinal bacteria are thought to contribute to the pathogenesis of many digestive diseases. However, studying the complex ecosystem at the human mucosal-luminal interface (MLI) is challenging and requires an integrative systems biology approach. Therefore, we developed a novel method integrating lavage sampling of the human mucosal surface, and high-throughput microbiome and proteomics, linked with a unique suite of bioinformatic and statistical analyses. Shotgun proteomic analysis of secreted proteins recovered from the MLI confirmed the presence of both human and bacterial components. Spectral data were subjected to a rigorous data processing pipeline to optimize suitability for quantitation and analysis, and then were evaluated using a set of biostatistical tools. Analysis of the metaproteome revealed a significant regional anatomic (biogeographic), age, and disease (inflammatory bowel disease) features. Six distinct protein communities within the metaproteome network were identified. Distinct protein communities were each associated with biogeography, age, and disease state. Moreover, the disease state changed the composition of some of the associated communities. Using a pyrosequencing and Illumina GA analysis of microbiome composition, an overlapping set of microbial communities were identified sharing these biogeographic, age, and disease features. Comparison of independent patient cohorts confirmed the conservation of these protein and microbial communities, which we term mucosal neighborhoods. *In situ* methods document the physical localization of mucosal neighborhoods at the intestinal surface. Hence, mucosal neighborhoods appear to reflect functional biologic communities relevant to the basic biology and disease processes at the MLI.

S69

Intestinal Microbiota Involved in Rheumatoid Arthritis. Scher J. NYU School of Medicine, New York, NY, United States.

Introduction: The etiology of RA remains unknown, but genetic and environmental factors have been implicated. An infectious trigger has been sought but conventional microbiologic techniques have been uninformative. The human intestine contains a dense, diverse and poorly characterized (~80% uncultured) bacterial population whose collective genome (microbiome) is 100 times larger than its human host. Several animal models are resistant to inflammatory arthritis under germ-free conditions but develop disease with introduction of gut commensal bacteria. Multiple lines of investigation also suggest a link between human RA and intestinal microbes. Methods: The NYU Microbiome Center for Rheumatology and Autoimmunity was established to study gut microbiota in RA and related conditions. A cross-sectional study and prospective proof-of-concept intervention trial are ongoing. Clinical data, blood and fecal samples are collected. To date, intestinal microbiomes have been analyzed in 35 new-onset, drug-naïve RA patients (NORA), 18 chronic established RA patients (CRA), 18 psoriatic arthritis (PsA) patients and 9 healthy controls (H). DNA was purified and variable 16s rRNA gene regions amplified to undergo pyrosequencing and phylogenetic classification. Results: A single species belonging to the Bacteroidetes phylum was significantly overrepresented in fecal microbiota from NORA patients (range 13%-67%; mean=38%) *vs.* CRA, PsA and H controls ($p<0.001$). Discussions: This is the first study using high-throughput technologies to assess intestinal microbiota in RA patients. Our preliminary data suggest that the initial phases of clinical autoimmune arthritis are associated with a particular gut microbiome signature. Thus, the intestinal microbiota merits further investigation as potential trigger for autoimmunity and clinical RA.

Symposium 16–The Multiplicity of DNA Polymerases: A Strategy for Maintaining Genome Stability

S70

Novel Roles for Specialized DNA Polymerases in Repetitive DNA Replication. Eckert KA. Pennsylvania State University College of Medicine, Hershey, PA, United States.

Repetitive DNA sequences constitute ~50 % of the human genome, yet have been understudied with regard to mutational mechanisms. Microsatellites are a type of interspersed repeat found ubiquitously throughout the genome and many are functional. We have shown that a high degree of mutational variation is present among microsatellites of differing sequence after replication in human cell lines. We examined the contribution of DNA polymerases to this mutational variation. The majority of DNA polymerases examined, including Polymerases δ and ε, produce microsatellite errors *in vitro* at a frequency that is up to 100-fold higher than the frequency of frameshift errors in exonic sequences. A significant exception to this rule is polymerase κ, which is unusually accurate during microsatellite DNA synthesis. Polymerases κ and η produced a high proportion of microsatellite stabilizing interruption errors. Biochemically, polymerase κ effectively competes with polymerase δ for DNA synthesis. Thus, polymerases δ and κ act cooperatively to carry out efficient microsatellite DNA synthesis and have complementary error rates that may enhance the fidelity of genome-wide DNA replication. Our study extends the requirement of Y family polymerases to maintain genome stability beyond accurate replication of DNA lesions, to include accurate synthesis of repetitive DNA sequences.

S71

Regulation of DNA Polymerase Eta in Human Cells by Post-Translational Modifications. Lehmann AR, Sabbioneda S, Goehler T, Chamberlain G. University of Sussex, Brighton, United Kingdom.

Translesion synthesis (TLS) is the major way in which human cells deal with damaged DNA during replication. TLS is carried out mainly by the Y-family of DNA polymerases. The Y-family DNA polymerase (pol) eta is the main polymerase for bypassing UV damage and is defective in the variant form of xeroderma pigmentosum. The catalytic domain is contained within the N-terminal two thirds of the protein, but sites of modification and interaction with other proteins are located close to the C-terminus. The UBZ ubiquitin-binding motif, bipartite NLS and PIP box PCNA-binding motif are all important for localisation of pol eta in replication factories during S phase and for efficient TLS. Pol eta can be post-translationally modified by both mono-ubiquitination and phosphorylation. Ubiquitination results in an intramolecular interaction between the UBZ of a pol eta molecule and the ubiquitin moiety attached to the same molecule. This appears to be a negative regulation to keep pol eta away from the chromatin in undamaged cells. Pol eta becomes phosphorylated at ser601on treatment with DNA damaging agents. This is mediated by the ATR protein kinase, and requires the UBZ motif of pol eta but not the PIP box. Phosphorylation of pol eta is important for complete restoration of normal TLS, survival and checkpoint activation in UV-irradiated xeroderma pigmentosum variant fibroblasts. In addition to binding to ubiquitin, we have recently found that pol eta also binds to the small ubiquitin-like modifier SUMO. We have tentatively identified a SUMO-interacting motif in the catalytic domain of pol eta.

S72

Function and Control of Translesion DNA Polymerases. Foti JJ[1], Devadoss B[1], Xie K[1], D' Souza S[1], Minesinger B[1], Winkler J[2], Doles J[3], Hemann MT[3], Collins JJ[2], Walker GC[1]. [1]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, United States, [2]Department of Biomedical Engineering and Center for Advanced Biotechnology, Boston University, Boston, MA, United States, [3]The Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA, United States.

Translesion DNA synthesis (TLS) carried out by specialized DNA polymerases is an important mechanism of DNA damage tolerance in all domains of life. In both bacteria and eukaryotes, the location and timing of TLS DNA polymerase function is controlled by complex sets of protein-protein interactions. Both bacterial DinB (DNA pol IV) and mammalian DNA pol kappa display a preferential ability to insert dC opposite N^2-furfuryl-dG. Our efforts to understand the basis of the lethality of DinB overproduction have led to new insights that have implications for the action of bactericidal antibiotics. In eukaryotes, the major mutagenic branch of TLS is carried out by Rev and DNA pol zeta (Rev3/Rev7) and is controlled in part through interactions between the Rev1, Rev3, and Rev7 proteins. The molecular mechanisms by which initially chemoresponsive tumors develop therapeutic resistance remain poorly understood. To test whether resistance to front-line therapy results from mutations introduced by TLS over lesions introduced by DNA damaging chemotherapeutic agents, we utilized a tractable model of B cell lymphoma. As expected, suppression of Rev1inhibits both cisplatin- and cyclophosphamide-induced mutagenesis in cultured lymphoma cells. By performing repeated cycles of tumor engraftment and treatment, we showed that Rev1 plays a critical role in the development of acquired cyclophosphamide resistance. Thus, chemotherapy not only selects for drug-resistant tumor population but also directly promotes the TLS-mediated acquisition of resistance-causing mutations. Using a preclinical model of lung adenocarcinoma to investigate whether the effect of impairment of TLS on the response of aggressive late-stage lung cancers to cisplatin, we found that suppression of Rev3 led to a significant extension in overall survival of treated mice.

S73

DNA Polymerase Dysregulation in Cancer and Animal Models. Wood RD. University of Texas MD Anderson Cancer Center, Smithville, TX, United States.

Mammalian genomes encode fifteen distinct DNA polymerases, devoted to aspects of replication, repair, or recombination of DNA. The polζ catalytic subunit REV3L participates in translesion DNA synthesis. Uniquely among specialized polymerases, REV3L is essential during mouse embryogenesis. Cells deficient in *Tp53* can survive *Rev3L* deletion, but are sensitive to DNA damage and have increased chromosome translocations. To determine whether polζ is necessary for proliferation of normal cells, primary mouse fibroblasts were established in which *Rev3L* could be conditionally inactivated by Cre recombinase. We found that even in the absence of externally-induced DNA damage, polζ is essential for proliferation of normal mammalian cells by preventing replication-dependent DNA breaks. *Rev3L* was conditionally deleted from tissues of adult mice to create a mosaic model using MMTV-Cre. Loss of REV3L was tolerated in epithelial tissues, but not in the hematopoietic lineage. Thymic lymphomas occurred sooner and with higher incidence in $Tp53^{-/-}$ mice when Rev3L was conditionally deleted. These lymphomas were usually composed of *Rev3L* null T-cells and were frequently oligoclonal, showing that loss of *Rev3L* favors events leading to tumor formation. In $Tp53^{+/+}$ and $Tp53^{+/-}$ mice, mammary tumors with deleted *Rev3L* arose earlier and with higher frequency than in control mice. These tumors had preneoplastic changes in adjacent tissue. Polζ therefore acts as a suppressor of spontaneous tumorigenesis.

S74

REV1 and DNA Polymerase ζ Maintain Genomic Stability by Promoting DNA Repair. Canman CE. University of Michigan, Ann Arbor, MI, United States.

REV1 and DNA Polymerase ζ (REV3 and REV7) play important roles in translesion DNA synthesis in which DNA replication bypasses blocking lesions. REV1 and Polζ are also implicated in promoting the repair of interstrand DNA crosslinks (ICLs) and DNA double stranded breaks (DSBs); however, the mechanisms by which they increase tolerance to ICLs and DSBs are poorly understood. I will present evidence that human REV1, REV3, and REV7 form a complex *in vivo* and together, promote homologous recombination (HR) repair. Cells lacking REV3 are hypersensitive to agents that cause ICLs or DNA DSBs, including the PARP inhibitor, olaparib. siRNA-mediated depletion of REV1, REV3 or REV7 leads to increased chromosomal aberrations, residual DSBs, and sites of HR repair following exposure to ionizing radiation consistent with a direct role in promoting HR repair. Previous studies have demonstrated an epistatic relationship between Polζ and genes encoding proteins comprising the Fanconi anemia (FA) core complex with regards to resistance to agents that create ICLs. Additionally, both FANCD2 and FANCI, play important regulatory roles in ICL and HR repair. We therefore investigated whether FANCD2 is epistatic to REV1 or REV3 in promoting gene conversion efficiency *via* HR repair. Our preliminary data suggests that this is the case and human REV1 and REV3 function epistatically to FANCD2 in cellular survival after ICL damage mediated by MMC. We are now proposing a model where replication fork stalling during HR repair DNA synthesis may be resolved through Polζ-dependent TLS, which is a process regulated by the FA pathway.

S75

DNA Polymerases in Meiosis. Sweasy JB, McDaniel K, Kidane D. Departments of Therapeutic Radiology and Genetics, Yale University School of Medicine, New Haven, CT, United States.

DNA polymerases have been shown to be important in a variety of DNA replication and repair contexts but little is known about the roles of DNA polymerases in meiosis. Our work focuses on the roles of DNA polymerase beta (Pol beta) during meiosis in the mouse. We have found that Pol ß is necessary for meiosis and that it needs to be present in order for SPO11 to be removed from the DNA ends. Our data also show that Pol ß is important for genome stability of the germline. Additional meiotic functions are also likely and these will be discussed, based upon preliminary data. The meiotic functions of other DNA polymerases during meiosis in the mouse will also be covered.

Symposium 17–Perspectives on the Evaluation and Interpretation of Mutagenicity and Genotoxicity Data

S76

Overview: Perspectives on the Evaluation and Interpretation of Mutagenicity and Genotoxicity Data. Sonawane B. U.S. Environmental Protection Agency, Washington, DC, United States.

Data interpretation from the mutagenicity or genotoxicity testing is an important aspect of hazard characterization. In particular, linking the genotoxicity data of xenobiotics (*e.g.*, environmental agents, pharmaceuticals, food additives, cosmetics ingredients) in target cells/tissues to carcinogenic potential in humans is essential. Such data are routinely used within a weight-of-evidence (WOE) approach by regulatory agencies and international organizations in assessing cancer risk. There are similarities and differences among these approaches particularly regarding data needs and in the interpretation of results for articulating WOE and ultimately assessing the potential of agents to cause mutagenicity/genotoxicity. Although the relationship between genotoxicity and carcinogenicity is well established, this relationship is complicated due to non-genotoxic mechanisms of carcinogenesis thereby making the relevance of data interpretation to human risk evaluation at times tenuous. The goal of this symposium is to present the current understanding, practices, and perspectives of select regulatory agencies and international organizations as well as viewpoints of industry and academia. Several case examples will be presented to illustrate the factors that influence data interpretation and their use in the cancer risk evaluation of xenobiotics. Disclaimer: The views expressed in this abstract are that of the author and does not represent and should not be construed to represent U.S. Environmental Protection Agency determination or policy.

S77

Overview of IARC and IRIS Use of Genetic Toxicity and Mechanistic Data. Cogliano VJ. U.S. EPA, Washington, DC, United States.

Genetic toxicity studies have been considered in carcinogen risk assessments for decades. Similar consideration has more recently expanded to a wider class of mechanistic studies. This presentation will give an overview of how genetic toxicity and mechanistic information are used in health hazard assessments at the IARC Monographs program and at the U.S. EPA's IRIS program. Instances where genetic toxicity or mechanistic information was pivotal to an assessment's conclusions will be highlighted, and overall trends will be examined. Both programs use criteria that are largely similar, although there are some differences that can be attributed to the different scopes of the two programs and to the different times that an assessment was completed by each program. The views expressed in this presentation are those of the author and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

S78

Dichloromethane: A Case Study for the Interpretation of Genetic Toxicity Data by IARC and the IRIS Program. Gibbons CF. U.S. Environmental Protection Agency, Washington, DC, United States.

Both the U.S. Environmental Protection Agency's (EPA) Integrated Risk Information System (IRIS) Program and the International Agency for Research on Cancer (IARC) develop toxicological assessments of human health effects that use genetic toxicity data when available to conduct hazard identification and to help inform the mode of action for the carcinogenic effects of a given chemical. Although both agencies have a similar goal in identifying environmental agents that can increase the risk of human cancer, there are often differences in the interpretation of mechanistic data. An example of a chemical that has had a differing interpretation by the two agencies is dichloromethane (methylene chloride). IARC determined that dichloromethane was possibly carcinogenic to humans based on inconsistent evidence of tumors in humans, while EPA determined that dichloromethane is likely to be carcinogenic in humans *via* a mutagenic mode of action. Dichloromethane has been shown to be genotoxic and mutagenic in numerous *in vitro* assays, but the data are less consistent *in vivo*, primarily due to interspecies differences in bioactivation *via* the glutathione-S-transferase pathway. These metabolic differences will be discussed in the context of interpreting the genetic toxicity data and the implications of a "yes or no" determination of a chemical's mutagenic potential. The views expressed in this presentation are those of the author and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

S79

Evaluation and Interpretation of Chromosome Aberration Data on Chromium Picolinate: A Dietary Supplement Ingredient. Gudi R. U.S. Food and Drug Administration, College Park, MD, United States.

Chromium picolinate (CrPic) is a stable covalent complex of trivalent chromium (Cr^{+3}) and picolinic acid. Chromium in the trivalent form occurs naturally in conventional foods (plants and animals) and is considered to be an essential trace element for humans with low toxicity. Similarly, picolinic acid is an endogenous metabolite of tryptophan metabolism is present in human tissues[1]. CrPic is widely included in dietary supplements, especially in multivitamin, multimineral products for several years[1]. It is promoted for its beneficial effects in weight loss and glucose control products. However, a number of publications have reported conflicting genotoxic effects of CrPic raising questions about its safety. Among several genetic toxicity studies found in the literature, chromosomal aberration (CA) tests have been used to evaluate the clastogenicity of CrPic in both *in vitro* and *in vivo* test systems of animals and humans. A review of *in vitro* chromosome aberration studies shows significant differences in study design, test article characterization, solvent selection, appropriate controls, criteria for top dose selection and the study results. These differences will be elaborated and the evaluation and interpretation of the data will be discussed. [1]Institute of Medicine, Food and Nutrition Board; National Research Council, Board on Life Sciences. Dietary Supplements: A Framework for Evaluating Safety. Washington, DC: The National Academies Press; 2004 Apr.

S80

Assessment of Mutagenicity and Carcinogenicity: An Overview of Recent Health Canada Research in Support of Hazard and Risk Assessment. White PA, Lemieux CL, Long AS, Yauk CL, Marchetti F, Douglas GR, Singer T. Health Canada, Ottawa, ON, Canada.

In 1986 the Department of Health and Welfare (now Health Canada) recognized mutagenicity as a *bona fide* toxicological endpoint and published guidelines for the evaluation of mutagenicity. The Canadian Environmental Protection Act (CEPA, 1988) necessitates mutagenicity assessment for all new chemicals introduced into the Canadian marketplace, and the required assessment tools include *in vitro* tests for mutagenicity and chromosome aberrations, and, if production volumes exceed 10 tonnes, an *in vivo* test for chromosome damage or gene mutation. Although cancer risk is a primary motive for such assessments, there is renewed recognition and appreciation of mutagenic hazard *per se*. The requirement for assessment of *in vivo* mutagenicity led to two decades of research on the establishment and validation of transgenic rodent (TGR) assays (*e.g.*, Muta^{TM}Mouse), and a new TGR OECD Test Guideline (TG 488). In addition, companion *in vitro* TGR tools based on cell lines (*e.g.*, FE1) and primary cells (*e.g.*, hepatocytes) have been developed to provide an opportunity for matched *in vitro* and *in vivo* assessments. Current research is employing these *in vivo* and *in vitro* TGR tools to study: (1) relationships among a wide range of endpoints; (2) novel *in vivo* mutagenicity endpoints; (3) dose- and concentration-response kinetics; (4) transgene mutations in germ cells; (5) comparative potency of complex mixtures; (6) non-concordance between other mutagenicity test results and carcinogenic activity; and (7) using toxicogenomics, mutagenic mode of action (MOA). This research, which is designed to facilitate more informed risk assessment, is closely linked to the needs of regulatory programs.

S81

European Union Requirements and Their Consequences for Consumer Product Genotoxicity Safety Testing. Pfuhler S. The Procter and Gamble Company, Cincinnati, OH, United States.

Regulations enforced within the European Union (EU) such as REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) and the 7th Amendment to the Cosmetics Directive continue to have big impact on the way global companies deal with genotoxicity safety testing. The 7th Amendment prohibits any acute *in vivo* genotoxicity tests for cosmetic ingredients, thereby triggering a search for innovative hazard and risk assessment concepts. The increasing desire to move away from or limit *in vivo* testing triggered a boost in research for animal-free assays, and in the scientific community efforts are increasing to replace *in vivo* tests by appropriate *in vitro* assays. This could be accomplished by improving the performance of the existing *in vitro* tests and the development of new *in vitro* assays with better predictive capacity which will be summarized in this presentation. The global need across many product categories for improved genotoxicity testing is underlined by the tens of thousands of substances being tested for the purposes of REACH registration which, using a classical testing scheme, could lead to a very high number of additional tests in animals. The limitations triggered by these and other regulations, as well as the sheer number of chemicals that need to be tested, call for more flexibility in how we assess genotoxic risk today. An attempt will be made to illustrate the difficulty associated with diverging requirements and a strategy will be suggested that was designed to allow for adaptation and flexibility.

S82

Evaluation and Interpretation of Genotoxicity Data: An Industry Perspective. Gollapudi BB. The Dow Chemical Company, Midland, MI, United States.

Tests for genotoxicity constitute a critical component of the safety assessment for new and existing chemicals. These tests have been generally effective in preventing the introduction of genotoxic agents into commerce and potential human exposure. For more than four decades, genotoxicity data have been interpreted in a qualitative binary mode, *i.e.*, either positive or negative with little or no emphasis on dose-response characterization or derivation of no-observed-genotoxic-effect levels (NOGELs). It often takes extensive additional experimental effort to follow-up on a single, often weak, positive finding with no assurance of adequate resolution. Hence, researchers have become reluctant to advance such a molecule through an R&D program or a registration process, given the difficulty of overcoming the stigma and challenges of a positive genotoxicity finding. This sometimes has the unintended consequence of discarding molecules of high societal value despite the existence of other toxicology data demonstrating low human risk. It is envisioned that a better understanding of the dose-response for genotoxicity through robust experimental designs can help generate useful quantitative data to better inform human risk assessment. It is further suggested that genotoxicity tests be designed and interpreted not in isolation but in the context of other available toxicology data including toxicokinetics as well as relevant human exposure information. Finally, it is imperative that positive results from short-term genotoxicity assays be carefully weighed along with other plausible mode(s) of action (MoA,) (*e.g.*, nuclear receptor activation, regenerative cell proliferation, altered hormonal homeostasis) prior to attributing a mutagenic MoA in carcinogenicity studies.

S83

The Use of Mutagenicity Determinations in Risk Assessment: An Academic Perspective. Eastmond DA. University of California, Riverside, CA, United States.

In assessing the risks associated with low dose exposure, national and international regulatory agencies frequently use mutagenicity determinations to inform their risk assessment decisions. While weight-of-evidence approaches are commonly used, the relative weights and outcomes of these determinations can vary from agency to agency. As part of a project to identify factors that influence risk assessment decisions, I have reviewed over 30 mutagenicity determinations made primarily by the U.S. Environmental Protection Agency's IRIS program but also evaluations by Environment Canada/Health Canada, the UK Committee on Mutagenicity, the European Commission, and the International Agency for Research on Cancer as well as other WHO expert groups. Similar conclusions have generally been reached by these authoritative bodies, but it is not uncommon for somewhat different outcomes to be reached. In addition to evaluating the specific *in vitro* and *in vivo* genotoxicity test results, other toxicological factors such as structural similarity to known mutagenic carcinogens, metabolism and toxicokinetics, distinctive mutations in cancer-related genes in animal tumors, high doses or levels of cytotoxicity, as well as the origin of the induced oxidative DNA damage were identified as playing important roles in the decision-making process. In this presentation, I plan to overview the mutagenicity evaluation process and use specific examples to illustrate how information on these modifying factors has been used by various agencies in making mutagenicity and risk assessment decisions. The recognition of factors that have played key roles in previous mutagenicity decisions should facilitate and provide consistency for future mutagenicity determinations.

Special Interest Group Meeting Abstracts

Applied Genetic Toxicology Special Interest Group

SG1

Further Characterization of the Genotoxic Effects of Phenolphthalein (PHT). Heard PL. Pfizer Global Research and Development, Groton, CT, United States.

PHT is known to induce tumors in rodents but is negative for mutations, DNA strand breaks and DNA adducts. Chromosome aberrations are associated with cytotoxicity and increases in micronuclei are only seen at very high doses. The majority of the micronuclei are kinetochore positive indicating aneuploidy. The mechanism that leads to the induction of chromosome loss by PHT was investigated. Image based assays using CHO cells, and flow cytometric assays using TK6 cells were performed to determine the potential cellular targets and cellular activities affected by PHT. A 24-27 hour direct test condition was chosen for the evaluation. The data for the following endpoints were collected; tubulin content, DNA content, centrosome enumeration, apoptosis, and mitotic inhibition. This presentation will discuss the outcome.

Environmental Genetic Toxicology Special Interest Group

SG2

National Center for Radioecology (NCoRE): A New Network of Excellence for Environmental Radiation Risk Reduction and Remediation. Lance S. Savannah River Ecology Lab, Aiken, SC, United States.

With the renewed and growing interest in nuclear energy, scientists from the Savannah River Site recognized a pressing need to build the pool of radioecology expertise in the United States and abroad. To address this need, in early 2011 the new National Center for Radioecology (NCoRE) was formed to serve as a network of excellence for environmental radiation risk reduction and remediation. The Savannah River National Laboratory manages NCoRE and will serve as the technical liaison with key partners and members. Currently the key U.S. member organizations include the University of Georgia Savannah River Ecology Laboratory (SREL), Duke University, Colorado State University, Oregon State University, and the University of South Carolina. In addition the Institut De Radioprotection Et De Surete Nucleaire (IRSN, France) and the Chernobyl Center's International Radioecology Laboratory (IRL, Ukraine) serve as key overseas partners. The academic and research specialties of each of the member institutions will provide the foundation for training the next generation of radioecologists. In particular, SREL will begin offering the Nuclear Regulatory Commission funded "GA/SC Regional Environmental Radiation Protection Curriculum." I will describe the rationale for the creation of NCoRE and our primary areas of research expertise. I will also discuss NCoRE's goals and opportunities to conduct research at SREL's Low Dose Irradiation Facility.

SG3

Nuclear Power Generation and Environmental Disasters: Lessons Learned from Chernobyl and Fukushima Daiichi. Dubrova YE. Department of Genetics, University of Leicester, Leicester, United Kingdom.

The year 2011 is a very special year for everyone involved in radiation research. Twenty five years ago on 26 April 1986, the largest reported release of radioactive materials occurred after the Chernobyl accident. As a result, many regions of Europe were heavily contaminated by nuclear fallout. In a first three months after the accident, acute radiation doses to humans occurred though external and internal exposure from iodine-131 with half-life of about 8 days. After the decay of unstable Iodine-131, exposure from more stable isotopes, mainly Caesium-137, became the main source of radiation risk for the population of contaminated regions. Ironically, 25 years after the Chernobyl disaster a similar accident occurred in the Fukushima Nuclear Power Plant in Japan. In my presentation, I will describe the sequence of events during the two accidents and compare the pattern of post-Chernobyl and post-Fukushima radioactive contamination and their potential effects on human health.

Risk Assessment Special Interest Group

SG4

The U.S. Tox21 Effort: Current Status. Witt KL. National Institute of Envirnomental Health Sciences, Research Triangle Park, NC, United States.

Moving toxicology from a predominantly observational science at the level of disease-specific models to a predominantly predictive science focused upon molecular target-specific, mechanism-based, biological observations requires the integration of assays that target key pathways, molecular events, or disease processes into a research and testing framework. The purpose of Tox21, a collaborative effort involving scientists from EPA, NCGC, NTP, and FDA, is to accomplish this goal. The Tox21 consortium consists of experts in cellular and molecular biology, toxicology, bioinformatics, chemoinformatics, and modeling. Tox21 has completed its initial proof-of-principle pilot stage, during which time over 100 quantitative high throughput screening (qHTS) assays were conducted on ~2800 compounds at the NCGC using 25 different cell types. In Tox21 Phase II, assays exploiting the qHTS approach, with an initial focus on nuclear receptors and stress response pathways, will be used to characterize the toxicity of a 10,000 compound library that broadly covers chemical space. This presentation will discuss the compounds and assays that have been selected for Tox21 Phase II, the rationale for their selection, and future plans.

SG5

Transcript Profiling to the Animal Cancer Phenotype: Maintaining the Gold Standard but Shortening the Cancer Bioassay. Waters MD. Integrated Laboratory Systems (ILS), Inc., Research Triangle Park, NC, United States.

The National Toxicology Program's rodent cancer bioassay is unquestionably the gold standard to which other short-term genotoxicity tests and toxicogenomics bioassays are compared. While short term tests may be predictive of carcinogenicity with few exceptions they do not serve in any way as surrogates for the 2-year bioassay. With the application of predictive and mechanistic toxicogenomics approaches to rodent cancer models the realization of a shorter-term rodent cancer bioassay is within reach. The question for discussion at this EMS Risk Assessment Special Interest Group is how to make the best use of rodent carcinogenicity data with the objective of strengthening its value while shortening the time required to perform the bioassay. The presentation will explore the application of key events determinations based on apical tests and amplified by dose-response toxicogenomics studies to yield defensible mode of action determinations and facilitate dose response assessments within a shorter bioassay time frame. Specific exemplary datasets and opportunities for further investigation will be discussed.

SG6

Abstract Not Available.

Epigenetics Special Interest Group

SG7

Challenges of QAQC in the Analyses of Epigenetic Markers in Human Studies. Holland N, Yousefi P, Aguilar R, Quach H, Volberg V, Barcellos L. School of Public Health, CERCH, University of California, Berkeley, CA, United States.

Epigenetic mechanisms, particularly DNA methylation, have attracted increasing interest in the etiology of disease, especially in regard to the hypothesized 'fetal origins of disease', and as a possible link between the genetic and environmental determinants of health. Methodologies for investigating DNA methylation have been rapidly emerging with increasing emphasis on genome-wide scale and throughput. The Illumina Infinium HumanMethylation BeadChips, both 27 & 450k, are currently the leading platforms for simultaneous measurement of DNA methylation at a high volume of sites across the genome. To apply these new methodologies effectively in large epidemiological studies, adequate quality assurance and control (QAQC) measures need to be implemented to minimize technical variability. While traditional sources of experimental variability still apply to data produced by high-throughput platforms, including chip, batch, and between laboratory variability, several additional considerations are unique to DNA methylation analysis, including bisulfite-conversion efficiency, tissue specificity of methylation and the effects of age and sex. We address the emerging QAQC challenges of high-throughput measurement of DNA methylation using samples drawn from a large longitudinal birth cohort. We measured DNA methylation in newborns and 9 year old boys and girls using both the Illumina Infinium HumanMethylation27K and 450K BeadChips. We provide a detailed characterization of data quality, including both technical and biological factors that contribute to DNA methylation variability. Development of a standardized assessment of highly-multiplexed DNA methylation data is necessary to ensure comparability and validity of data generated across laboratories and platforms.

New Technologies Special Interest Group

SG8

Cutting-Edge Sequence Discovery of Human Genetic Variation. Gibbs RA. Baylor College of Medicine, Houston. TX, United States.

The discovery and monitoring of human mutagenic events is greatly enhanced by next generation sequencing. Using these methods we have uncovered more about the spectrum of germ line variation the human population, as well as the range of somatic variation that arises in cancer. The new methods are limited however by high primary error rates and the dependency on amplification methods. As a result, while we have expanded our knowledge in this arena, there is more to discover, as soon as we have the technologies in place.

Women in the EMS Special Interest Group

SG9

Women As Leaders in the Scientific Enterprise. Richmond G. University of Oregon, Eugene, OR, United States.

Strong leadership in science and technology is critically important in sustaining life on this planet and assuring the health and well-being of its inhabitants. These leadership roles are broad and diverse, from the instructor that teaches and nurtures the emerging scientist, to the director of a research group or laboratory, to the federal agency worker or political appointee who assists in setting policy and funding national priorities. Women scientists and engineers often are at a disadvantage in attaining these leadership positions because of lack of mentorship or knowledge of professional skills that can move them up the career ladder. In this presentation I will provide an overview of some of the tactics and techniques that we teach in Leadership and Negotiation workshops developed by COACh, a grass-roots organization of women scientists and engineers in the U.S. that is working to level the playing field for women in technical fields. Over 6000 women scientists and engineers in the U.S. have participated in these workshops over the past year and with measurable benefits to their career trajectories.

Molecular Epidemiology Special Interest Group

SG10

An Overview of ChIP-chip Technology and Its Application in the Discovery of Thyroid Hormone Receptor Binding Sites in the Mouse Genome. Paquette M[1,2], Dong H[1], Gagné R[1], Williams A[1], Wade M[1], Yauk C[1,2]. [1]Environmental Health Sciences and Research Bureau, Healthy Environments and Consumer Safety, Health Canada, Ottawa, ON, Canada, [2]Department of Biology, Carleton University, Ottawa, ON, Canada.

Large numbers of proteins function through direct or indirect interactions with DNA. Chromatin immunoprecipitation can be used in combination with DNA microarrays (ChIP-chip), or with next generation sequencing (ChIP-seq), to characterize on a genomic scale, the DNA sequences that mediate regulatory control for a specific protein. ChIP-chip involves cross-linking DNA with proteins, sonicating the DNA to produce small DNA fragments, and immunoprecipitation of the protein-DNA complex using an antibody specific to a protein of interest. Enriched DNA is labelled and hybridized to microarrays. Hybridization signals from enriched samples (immunoprecipitated) are compared to labelled non-enriched DNA samples (total input) to determine sites of protein binding enrichment. In ChIP-seq, the enriched and total input samples are analyzed using next generation sequencing. In our laboratory, ChIP-chip is being used to profile nuclear receptor binding sites. Perturbations in the interaction between nuclear receptors and their genomic targets can greatly impact overall cellular function. The focus of our research is to characterize the thyroid hormone (TH) induced transcriptome to understand the molecular consequences of toxicant-induced hypothyroidism. THs bind to thyroid receptors (TRs) to control target gene expression. Disruption of TH physiology during critically sensitive periods in development can lead to adverse outcomes. We have identified TR binding sites and target genes using a ChIP-chip approach in juvenile euthyroid mice. The analysis has revealed novel TH responsive genes. We were also able to show that mutation of the DNA binding sites for select newly identified binding sites lead to disruption of TR-DNA interaction.

SG11

MicroRNAs: Biology, Technology, and Toxicology. Bourdon JA. Health Canada, Ottawa, ON, Canada.

MicroRNAs (miRNAs) are short (~22 nucleotide long), highly conserved, non-coding RNAs that are important post-transcriptional regulators of mRNA levels. MiRNAs operate through binding to the 3' untranslated regions of target mRNA, leading to degradation or translational repression. Individual miRNAs have been shown to repress hundreds of target genes. Moreover, miRNAs are known to affect at least one third of all human genes. The increasing amount of information available on miRNA targets and modes of action has enabled researchers to link specific miRNAs to various important biological processes. Altered miRNA profiles have also been associated with a number of diseases, including cancer, diabetes, neurological diseases and viral infections. As such, miRNAs are considered potentially important biomarkers of exposure and effect. This talk will focus on the biology behind miRNAs, the experimental applications employed to identify differentially expressed miRNAs and their mRNA targets, and the biological implications of changes in miRNA abundance. The techniques reviewed will include genomic approaches (miRNA arrays and sequencing) to identify up- and down-regulation of miRNAs, miRNA target prediction and pathway identification techniques, as well as *in vitro* approaches used to identify biological miRNA effects (transfection of mimics and inhibitors). Demonstrations on the application of these approaches will be given using work conducted as part of Health Canada's initiative to apply systems biology approaches to investigate mechanisms of action and risk of adverse health outcomes following exposure to toxic chemicals. This will include work on nanoparticle exposures, including carbon black and titanium dioxide, and the importance of miRNAs 135b, 146a and 146b in resolution of particle induced inflammation. Finally, literature on the use of miRNAs as potential serum biomarkers of effect and exposure will be reviewed.

Platform Abstracts

Platform Session 1–DNA Repair

1

Oligonucleotide Retrieval: A Versatile Technology for Calibrating the Fidelity of DNA Synthetic Processes *In Vivo*. Shen JC[1], Fox EJ[1], Loeb LA[1,2]. [1]Department of Pathology, University of Washington, Seattle, WA, United States, [2]Department of Biochemistry, University of Washington, Seattle, WA, United States.

We have established protocols for introducing biologically-derived oligonucleotides into cells, retrieving these substrates and determining errors in DNA synthetic processes. Each oligonucleotide harbors a 5'-biotin terminus and appropriate 3'- blocks with terminal non-hydrolysable nucleotides to prevent degradation by cellular nucleases. After transfection of double-stranded DNA into human cells, we can recover >50,000 molecules/cell. After transfection of single-stranded DNA molecules hybridized to a 5'-biotin labeled primer, extended primers are recovered on streptavidin beads; the frequency of mis-incorporation into the newly synthesized DNA varies from 10^{-4} to 10^{-6} and is likely dependent on the genetic background of the transfected cells. Counterintuitively, the error frequency of copying these templates is higher in nuclei, 10^{-3} to 10^{-5}, presumably due to error prone DNA polymerases. We have also measured the frequency of mis-incorporation using templates containing alternative DNA structures and modified nucleotides, as well as the incorporation of nucleotide triphosphate analogs. Further modifications of the assay allow measurements of the accuracy of end-joining and copying structures mimicking Holliday junctions and replication forks. The versatility of this assay will facilitate studies on the efficiency of DNA repair, the role of different human DNA polymerases in DNA synthesis and bypass of DNA damage, and permit stratification of individuals with respect to DNA repair capacity and fidelity of DNA synthesis. Enhancing the mutation frequency of cancer cells potentially represents a new avenue for treatment, particularly for patients with tumors that have accumulated large numbers of mutations and/or failed extensive chemotherapy.

2

Comet-Fish to Sensitively Assess Global and Transcription-Coupled Repair of DNA Lesions. Spivak G, Guo J, Hanawalt PC. Stanford University, Stanford, CA, United States.

We are developing the sensitive Comet-FISH assay, that combines single cell electrophoresis with fluorescence *in situ* hybridization, to measure the incidence and repair of low, physiologically relevant levels of DNA lesions, both in the genome overall and in defined DNA sequences, in wild type and repair-deficient human cells. We have designed and synthesized fluorescent probes for the transcribed and the non-transcribed strands of the p53 gene. The probes are labeled with Alexa 488 and Alexa 594, which emit green and red fluorescence, respectively. We record the appearance of signals corresponding to the complementary strands of the p53 gene in the "heads" and/or the "tails" of the Comets. Repair in the bulk of the DNA counterstained with DAPI is calculated from the relative amount of signal in the Comet tails. Our results should indicate whether the expressed p53 gene is repaired at a different rate than the genome overall, and whether the two DNA strands are repaired at similar or dissimilar rates. It is generally accepted that mammalian chromatin is arranged with DNA loops tethered to a matrix; when intact, the loops are tightly supercoiled; one single-strand break is sufficient to release superhelical tension within the loops, which then extend away from the nucleus. We are also attempting to elucidate how a single strand break affects the electrophoretic migration of the complementary DNA strand in a defined locus within a loop of unwound DNA, in contrast to that for the supercoiled, unbroken DNA loops that remain in Comet heads.

3

Chemotherapeutic Alkylation Treatment: Tumor Cell Response. Kim S, Williams KJ. University of Toledo College of Medicine, Toledo, OH, United States.

Tumor cells that have proficient mismatch repair (MMR) but lack methylguanine methyltransferase (MGMT) are highly susceptible to initial treatment of alkylating agents at chemotherapeutic levels. These cells undergo a prolonged second cell cycle, culminating in an aberrant mitotic phase and cell death (Schroering *et al. Cancer Res* 2009;6307-14). Apoptosis *via* classical caspase cleavage pathway is absent or only weakly activated in G_2/S phase of the 2nd cell cycle. In contrast, Apoptosis Inducing Factor (AIF) translocates to the nucleus to initiate an alternate route to cell death. A small fraction of exposed tumor cells consistently evade death. All clones developed from the resistant cells exhibit decreased hMutSa activity, increased mutator phenotype and chromosomal aberrations. Ongoing studies have demonstrated that resistant tumor clones have permanently decreased MMR activity due to decreased hMSH6, and increased alkylation resistance. All resistant tumor clones remain MGMT negative. In conclusion, decreased expression of hMSH6 leading to MMR deficiency is a common positive selection factor for emergence of tumor cells resistant to alkylating chemicals. Additionally, tumor cells succumbing to alkylation chemotherapy do not undergo the classical caspase cleavage route, instead these cells use the alternative AIF pathway. We are currently investigating cellular mechanisms leading to survival as well as the AIF death pathway as potential targets for therapy. Acknowledgements: The work was supported by NIH CA10657 (KJW).

4

Alkylation Sensitivity Screens Reveal a Cross-Species Functionome. Svilar D[1], Brown A[1], Tang JB[1], McDonald PR[1], Shun TY[1], Wang XH[1], Lazo JS[1], Begley TJ[2], Sobol RW[1]. [1]University of Pittsburgh, Pittsburgh, PA, United States, [2]University at Albany, State University of New York, Rensselaer, NY, United States.

DNA alkylating agents are toxic, carcinogenic, and teratogenic. Humans are exposed to alkylators in food, air, water, drugs, tobacco smoke and metabolic byproducts. Because of the ubiquitous nature and detrimental effects of alkylators, understanding their repair and resistance mechanisms is essential. To discover resistance genes to the alkylator temozolomide (TMZ), we conducted a synthetic lethal screen with TMZ and an siRNA library using a TMZ-resistant cell line (T98G). Genes satisfying the selection criteria were analyzed for biological pathway enrichment with NIH DAVID, Princeton GO term finder, and Ingenuity Pathway Analysis. DNA glycosylases specific for the repair of oxidative DNA lesions were highly enriched in all analysis programs. These hits were validated to sensitize cells to alkylation damage in cell lines with stable knockdown using unique lentiviral shRNA vectors. The knockdown cell lines were characterized by quantifying mRNA by qRT-PCR, protein levels by immunoblot and functional DNA repair capacity by a novel real-time molecular beacon DNA glycosylase assay. Furthermore, we find that similar biological processes were significantly enriched to sensitize bacteria, yeast and human cells to alkylators, suggesting that response and resistance to alkylation damage is evolutionarily conserved. Based on these results, we targeted a second conserved biological process and determined that knockdown of protein modification genes sensitized human and yeast cells to alkylators, further confirming our screening results. Our preliminary results suggest that conserved diverse biological processes impact alkylation sensitivity. Genes belonging to these processes represent new genes which modulate alkylation survival.

5

Structure and Substrate Specificity of a Mouse NEIL3 Ortholog. Liu M[1], Imamura K[1], Averill A[1], Jaruga P[2], Zhao X[3], Burrows CJ[3], Dizdaroglu M[2], Doublié S[1], Wallace SS[1]. [1]Department of Microbiology and Molecular Genetics, The Markey Center for Molecular Genetics, University of Vermont, Burlington, VT, United States, [2]Chemical Science and Technology Laboratory, National Institute of Standards and Technology, Gaithersburg, MD, United States, [3]Department of Chemistry, University of Utah, Salt Lake City, UT, United States.

To protect cells from oxidative DNA damage and mutagenesis, organisms possess multiple DNA glycosylases to recognize damaged bases and to initiate the Base Excision Repair (BER) pathway. Three DNA glycosylases have been identified in mammals that are homologous to the *Escherichia coli* Fpg and Nei proteins, Neil1, Neil2 and Neil3. In this study, we report the substrate specificity of Mus musculus (house mouse) Neil3 (MmuNeil3). In duplex DNA, MmuNeil3 recognizes the oxidized purines, spiroiminodihydantoin (Sp), guanidinohydantoin (Gh), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG) and 4,6-diamino-5-formamidopyrimidine (FapyA), but not 8-oxo-7,8-dihydroguanine (8-oxoG). Interestingly, MmuNeil3 prefers lesions in single-stranded DNA and in bubble structures. We determined the crystal structure of the glycosylase domain of MmuNeil3 (MmuNeil3Δ324). Although the structure of MmuNeil3Δ324 exhibits the same overall fold as other Fpg/Nei proteins, it has two distinct features. First, MmuNeil3Δ324 lacks the loop corresponding to the "αF-β9/10 loop" in Fpg proteins, which has been reported to stabilize the flipped-out 8-oxoG in the active site. Second, MmuNeil3Δ324 lacks the canonical void-filling residues, which have been shown to intercalate in the DNA helix and interact with the base opposite the lesion. These distinct structural features of MmuNeil3 provide insights into Neil3's substrate specificity. Acknowledgements: This work was supported by NIH Grant P01 CA098993 awarded by the National Cancer Institute.

6

Sumoylation of Ntg1 and the Regulation of Base Excision Repair in *Saccharomyces cerevisiae*. Swartzlander DB[1], Powers HR[1], Corbett AH[1], Doetsch PW[1,2]. [1]Emory University, Department of Biochemistry, Atlanta, GA, United States, [2]Winship Cancer Institute, Atlanta, GA, United States.

Oxidative DNA damage is the most frequently occurring DNA damage and arises in cells due to oxidative stress caused by environmental exposures and cellular metabolism. The base excision repair (BER) pathway initiated by N-glycosylase apurinic/apyrimidinic lyase proteins is primarily responsible for repair of oxidative DNA damage in both nuclei and mitochondria. Little is known about the effect post-translational modifications have on BER proteins and whether these modifications occur in response to cellular stress to regulate this evolutionarily conserved repair pathway. The *Saccharomyces cerevisiae* BER protein, Ntg1, is sumoylated (small ubiquitin-like modifier). Ntg1 was used as a model system to ascertain the effects sumoylation has on the regulation of BER in addition to understanding the dynamics of sumoylation in response to cell stress. In an effort to define the function of Ntg1 sumoylation, the mechanism of its sumoylation was determined. Our results show that sumoylation of Ntg1 increases in response to oxidative stress, is associated with nuclear localization, and requires the E3 ligases Siz1/Siz2 to generate both monosumoylated and multisumoylated Ntg1. Mutational analysis of putative Ntg1 sumoylation sites reveals that Ntg1 is predominantly sumoylated at five distinct consensus sumoylation sites which cluster at both termini with K396 being the major site of monosumoylation. Collectively, these results detail a biological pathway commencing with oxidative stress signaling and concluding in the post-translation modification of a key BER protein, providing insight into not just an important mechanism of regulating BER but also into the dynamics of sumoylation. Work supported by NIH Grant ES 011163.

7

Radiation Mitigators Yel1 and Yel2 Inhibit Radiation-Induced Error-Prone Microhomology-Mediated Recombination. Scuric Z, Farabi N, Schiestl RH. David Geffen School of Medicine at UCLA, Los Angeles, CA, United States.

DNA DSBs induced by ionizing radiation are severe forms of DNA damage, and result in cell death if not repaired. In mammalian cells DSBs are mostly repaired by direct ligation of broken ends through nonhomologous end-joining, a repair pathway that maintains genomic stability and suppresses tumorigenesis. Also, DSBs are repaired by microhomology-mediated recombination based on a few base pairs of homology between broken DNA ends, an error-prone DNA repair pathway known to contribute to genomic instabilities. Microhomology is often present at the junctions of radiation-induced genomic rearrangements, suggesting a possible link between radiation-induced microhomology-mediated recombination and radiation-induced carcinogenesis. We have previously shown that irradiated mammalian cells repair newly introduced extrachromosomal linearized plasmid substrates more frequently by microhomology-mediated recombination than non-irradiated cells. We also found that this increase in microhomology-mediated recombination is radiation-dose dependent. Further, we tested two Yel mitigators and a Tempol for their potential role in the prevention of microhomology-mediated recombination after radiation. Our data show that Yel 1 and Yel 2 but not Tempol significantly inhibit radiation-induced microhomology-mediated recombination events when applied two hours after ionizing radiation and transfection of linearized plasmid substrates. Based on our current data we can expect that Yel mitigators could be able to reverse spontaneous as well as radiation-induced microhomology-mediated recombination in cancer cells. We suggest that Yel mitigators will act as general anticancer agents by turning off continued genetic instability in other spontaneous cancer cells.

8

FANCJ Functions in Error-Free UV DNA Repair. Guillemette SS, Peng M, Cantor SB. University of Massachusetts Medical School, Worcester, MA, United States.

FANCJ is a breast cancer tumor suppressor that encodes a DNA helicase functioning in DNA repair. FANCJ directly interacts with the C-terminus of BRCA1 to facilitate DNA double strand break repair. More recently, a novel FANCJ function was revealed when FANCJ was uncoupled form BRCA1. The unbound FANCJ reduced HR and enhanced DNA damage tolerance through the translesion synthesis (TLS) polymerase Pol eta. As Pol eta promotes error-free UV bypass, we reasoned that FANCJ could also be essential for this process. Consistent with this idea, FANCJ has a dynamic response to UV damage including an induced interaction in chromatin with the monoubiquitinated form of PCNA and TLS factors RAD18 and Pol eta. FANCJ deficiency also directly impacts the ability of a cell to resolve UV lesions. Not only do UV induced lesions persist, but the mutation frequency is also dramatically enhanced implicating that FANCJ is essential for error-free UV lesion processing. Future studies may reveal FANCJ tumor suppressor functions in skin cancers in addition to its role in suppressing breast cancer.

9

Unified Model for Fanconi Anemia and Breast Cancer Gene-Network in Protecting Stalled DNA Replication Forks. Schlacher K[1,2], Jasin M[1]. [1]Memorial Sloan-Kettering Cancer Center, New York, NY, United States, [2]University of California, Los Angeles, Los Angeles, CA, United States.

Introduction: Breast cancer (BRCA) and Fanconi Anemia (FA) suppressor genes are critical for maintenance of genomic integrity when DNA replication is impeded. BRCA1 and BRCA2 are critical to the repair of double-strand breaks. Yet, FA-patient cells are not severely defective in break repair and the functional relationship between the FA/BRCA proteins during replication stalling remains enigmatic. Methods: We employed a DNA combing strategy with single molecule resolution to investigate the role of FA/BRCA proteins during perturbed replication, in addition to cellular and cytological assays monitoring genomic stability, break repair and survival. Results: We report that nascent replication tracts created before fork stalling with hydroxyurea are degraded in the absence of BRCA1 and BRCA2 but are stable in wild-type cells. Moreover, defects in monoubiquitination of FANCD2 lead to fork degradation, while BLM helicase, which also interacts with the BRCA/FA pathway, is not involved in fork stabilization and acts downstream during replication restart. Mutational analysis reveals that BRCA2-mediated fork protection is achieved by stabilizing RAD51 filaments, which is dispensable for DSB repair. BRCA2 prevents chromosomal aberrations upon replication stalling, which are alleviated by inhibition of MRE11, the nuclease responsible for this novel fork instability. Discussion: The data reveals a novel repair-independent function for the FA/BRCA proteins in protecting stalled replication forks from degradation by the MRE11 nuclease. Our collective results provide a unified molecular mechanism for a diverse set of proteins to maintain genome integrity at stalled forks and suggest maintenance of fork stability constitutes a novel tumor suppression mechanism.

Platform Session 2–Mutagenesis and Carcinogenesis Mechanisms

10

Subchronic Oral Exposure to Benzo[a]Pyrene Induces Changes in Gene Expression Associated with Cellular Transformation in Mouse Lungs: A Toxicogenomics Study. Labib S[1], Williams A[2], Lemieux CL[2], White PA[2], Halappanavar S[2]. [1]University of Ottawa, Ottawa, ON, Canada, [2]Health Canada, Ottawa, ON, Canada.

Benzo[a]Pyrene (BaP) is an environmental mutagen that alters the expression of genes involved in several biological processes, including AHR-response. The primary route of BaP exposure is *via* oral ingestion. Oral BaP exposure, however, does not result in hepatocarcinogenicity, but does cause lung cancer. In the present study, we examine pulmonary mRNA expression changes following subchronic exposure to BaP to understand molecular mechanisms that selectively prime the lung for carcinogenic transformation. Adult male Muta^{TM}Mice were exposed to BaP (25, 50, or 75mg/kg body weight/day) or to vehicle-control by oral gavage daily for 28 days and sacrificed 3 days post-exposure. DNA-adducts and lacZ mutant frequency was measured in lung tissue. Using whole-genome oligonucleotide microarrays, pulmonary mRNA expression was analysed. Results were validated using pathway-specific PCR arrays. Expression profiles in lungs were compared to profiles in livers derived from same mice. A 240 to 600-fold increase in DNA-adducts and a 5 to 18-fold increase in lacZ mutant frequency were observed in lungs in a dose-dependent manner compared to matched controls. Overall, 423 genes were significantly differentially expressed (fold-change>1.5 and FDR-adjusted p-value<0.05). These genes were mainly associated with DNA repair, cell proliferation, apoptosis, angiogenesis and calcium homeostasis pathways. Here we demonstrate that lung is more responsive to oral BaP exposure than liver. The adverse outcome pathways identified are linked to carcinogenic processes and were specifically altered in the lung tissues. Implication of such selective alterations in lungs following chronic BaP exposure will be discussed.

11

The Mouse Diversity Genotyping Array Profiles Tissue- and Genotype-Specific Mutations across the Mouse Genome. Eitutis ST, Wishart AE, Hill KA. The University of Western Ontario, London, ON, Canada.

Approaches for the study of spontaneous and induced mutations have employed endogenous gene and transgene targets with great success, but detailed study of the origins, mechanisms and impact of mutations might only be accomplished with a genome-wide perspective. The leap to whole genome sequencing for mutation detection is challenging from a bioinformatics perspective and cost prohibitive for most laboratories. The recently developed Mouse Diversity Genotyping Array (MDGA) provides a mutation target with a genome-wide representation. The single nucleotide polymorphisms (SNPs) represented on this array permit mutation detection at 623,124 sites and a 5- to 15-fold greater mutation target considering the possibility for “off target” mutation detection for 5 and 15 nucleotides on the 25mer probe surrounding each represented SNP. Herein, we applied the MDGA for the first assay of somatic mosaicism between spleen and cerebellum for two mice that are models of premature aging and two wild-type littermates. Apoptosis-inducing factor-deficient harlequin (hq) mice mimic premature aging with mitochondrial dysfunction and neurodegeneration. Significant somatic mosaicism was evident in three out of the four comparisons of cerebellar and splenic DNA ($p<0.001$). Putative mutations were not uniformly distributed across the chromosomes of the mouse genome in both spleen and cerebellum DNA samples ($p<0.001$). Mutation frequency was significantly higher in hq mice and putative mutations were over-represented on chromosomes 12, 13, and 14 in both spleen and cerebellum compared to wild-type littermates ($p<0.001$), suggestive of a mutation hotspot or signature. We demonstrate an affordable, high-throughput mutation profiling approach also applicable to environmental mutagen assessments.

12

DNA Structure-Induced Genetic Instability in Mammals. Wang G, Vasquez K. University of Texas at Austin, Austin, TX, United States.

Naturally occurring DNA repeat sequences, which are abundant in mammalian genomes, can adopt non-canonical (or non-B) DNA structures. We have found that two types of non-B DNA structures, H-DNA and Z-DNA, are intrinsically mutagenic in mammals. We found that an endogenous H-DNA-forming sequence from the human c-MYC promoter and a model Z-DNA-forming CpG repeat, induced mutation frequencies ~20-fold over background, largely in the form DNA double-strand breaks (DSBs). Characterization of the mutants revealed microhomologies at the breakpoints, consistent with a non-homologous end-joining repair of the DSBs (Wang & Vasquez, *PNAS*, 2004; Wang *et al.*, *PNAS*, 2006; Kha *et al.*, *JMB*, 2010). We have constructed novel transgenic mutation-reporter mice containing these human H-DNA and Z-DNA-forming sequences, to determine their effects on genomic instability in a chromosomal context in a living organism (Wang *et al.*, *JNCI*, 2008). Initial results suggest that both H-DNA and Z-DNA are mutagenic on mouse chromosomes, consistent with our initial findings using cell lines. Our current studies are designed to determine the role(s) of mammalian helicases, DNA and RNA polymerases, and repair enzymes in H-DNA and Z-DNA-induced genetic instability in mammalian cells. Our findings suggest that both H-DNA and Z-DNA, which have been reported to correlate with chromosomal breakpoints in tumors, are sources of genetic instability, and demonstrate that naturally occurring DNA sequences are mutagenic in mammalian cells and may contribute to disease etiology and evolution.

13

Identification of Different Mechanisms of Non-Genotoxic Carcinogens Based on Gene Expression Profiling in Primary Mouse Hepatocytes. Schaap MM[1,2], Jonker MJ[3], Zwart PE[1], Wackers P[3], van de Water B[4], Breit TM[3], Schoonen WG[5], Polman J[5], van Steeg H[1,2], Luijten M[1]. [1]National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands, [2]Department of Toxicogenetics, Leiden University Medical Center, Leiden, Netherlands, [3]MicroArray Department and Integrative Bioinformatics Unit, University of Amsterdam, Amsterdam, Netherlands, [4]Leiden Amsterdam Center for Drug Research (LACDR), Leiden University, Leiden, Netherlands, [5]Merck Sharp & Dome, Oss, Netherlands.

Under REACH, the European Community's new chemicals policy, the testing strategy for carcinogenicity is generally based on a combination of *in vitro* and *in vivo* genotoxicity assays. A lifetime cancer bioassay is only performed for substances having production volumes over 1,000 tonnes per annum and/or when compounds scored positive in the genotoxicity tests. Given that non-genotoxic carcinogens are negative for genotoxicity, they will not be detected under REACH. Ten to twenty percent of the known, probable or possible human carcinogens appears to be non-genotoxic. For one third of these compounds, exposure is high enough to expect a significant increased cancer risk. Therefore, alternative test systems to detect non-genotoxic carcinogens are urgently needed. We investigated whether primary mouse hepatocytes in combination with the toxicogenomics technology are useful to identify non-genotoxic carcinogens, having different modes of action (MOA). Primary mouse hepatocytes are well-studied and are known to maintain metabolic activity. We found multi-gene expression signatures in primary hepatocytes exposed to twenty different non-genotoxic carcinogens. These different, but specific, gene expression patterns could, in many cases, be linked to know MOAs of the tested non-genotoxic carcinogens. We believe that our approach is useful to identify MOAs of unknown substances and, as such, might become a valuable tool for risk assessment.

14

Tissue Specificity of Aristolochic Acid-Induced Carcinogenesis Examined by ACB-PCR Quantification of H-*Ras* Codon 61 CTA Mutant Fraction. Wang Y[1], McKim KL[1], Myers MB[1], Arlt VM[2], Parsons BL[1]. [1]National Center for Toxicological Research, U.S. FDA, Jefferson, AR, United States, [2]Institute of Cancer Research, Sutton, Surrey, United Kingdom.

Aristolochic acid (AA) is a strong cytotoxic nephrotoxin and carcinogen, associated with development of urothelial cancer in humans. AA induces forestomach, kidney and urothelial tract tumors in rats and mice. This study sought to gain mechanistic insight into AA-induced tissue-specific carcinogenesis through analysis of a tumor-relevant endpoint. Female Hupki mice (n = 3) were treated daily with 5.0 mg AAI/kg body weight by gavage, for 3, 12, or 21 days. Levels of H-*Ras* codon 61 CAA→CTA mutation were measured in mouse kidney (target organ) and liver (non-target organ) by allele-specific competitive blocker-PCR (ACB-PCR) because H-*Ras* codon 61 CAA→CTA mutation had been found previously in AA-induced rodent forestomach tumors and A→T transversion is the predominant type of mutation induced by AA. Statistically significant treatment-related differences were observed, with the H-*Ras* mutant fraction (MF) of mouse kidney exposed to 5.0 mg AAI/kg body weight for 21 days significantly higher than that of controls (Mann Whitney Rank Sum Test, $P < 0.05$). Further, statistically-significant correlations between AA-induced DNA adduct levels (previously measured in the same animals) and H-*Ras* MF measurements were evident in the 21-day exposure data (linear regression, $P < 0.05$). The significant increase in H-*Ras* codon 61 CTA MF observed in kidney, along with the correlation between DNA adducts and oncogene mutations, add to the weight of evidence that AA operates through a directly mutagenic mode of action. Also, these data indicate measurement of tumor-associated mutation is a useful tool for elucidating the mechanisms underlying the tissue-specificity of carcinogenesis.

15

Toxicogenomic Late Effects of Antineoplastic Therapies for Lymphomas. Marcondes JPC[1], Torres BP[1], Niéro-Melo L[1], Gaiolla RD[1], Luisi FAV[2], Salvadori DMF[1]. [1]São Paulo State University, Botucatu, SP, Brazil, [2]São Paulo Federal University, São Paulo, SP, Brazil.

Lymphomas are a heterogenous group of malignancies that arise in nodal sites with or without extranodal involvement. The treatment, based on polychemotherapy associated or not with radiotherapy, has provided high cure rates. However, it is known that such therapies can induce genetic mutations that could be related to development of second malignancies. Therefore, the present study aimed to evaluate the late effects of antineoplastic therapies for lymphomas. The relationship between DNA repair genes polymorphisms (XRCC1 codons 280 and 399, hOGG1 codon 326) or gene expressions with DNA damage and repair capability were investigated. Three groups were included in the study: pre-therapy, with 14 patients newly diagnosed with lymphoma and before any antineoplastic treatment; post-therapy, 29 patients with history of lymphoma, but currently negative for the disease, who had finished treatment at least three years before blood collection; control, 29 healthy subjects matched for age, sex and smoking habit. Results showed that patients at pre- and post-therapy presented higher amount of DNA damage and lower XRCC1 and hOGG1 expression than healthy subjects. Moreover, post-therapy group presented reduced DNA repair capability in relation to controls. Gene polymorphisms analysis demonstrated increased DNA repair capability in those subjects with $XRCC^{399arg/arg}$, $XRCC1^{280arg/his}$ and $hOGG1^{326ser/ser}$ genotypes. In conclusion, our data demonstrated that lymphomas were associated with high level of DNA damage. An late effect of therapies was observed on DNA repair capability of post-therapy patients. Therefore, even with negative diagnosis, patients after therapy might have increased risk for developing genetic-related diseases.

16

Functional Studies of Single-Nucleotide Polymorphic Variants of Human Glutathione Transferase T1-1 Involving Residues in the Dimer Interface. Josephy PD[1], Pan D[1], Ianni MD[1], Mannervik B[2,3]. [1]University of Guelph, Guelph, Canada, [2]Uppsala University, Uppsala, Sweden, [3]Stockholm University, Stockholm, Sweden.

Glutathione transferase T1-1 catalyses detoxication and bioactivation processes in which glutathione conjugates are formed from endogenous and xenobiotic substrates. Halogenated alkanes are metabolized to mutagenic species by the action of this enzyme. Examples of compounds activated by GST T1-1 include ethylene dibromide, a pesticide and widespread environmental contaminant, and bromodichloromethane, a byproduct of water chlorination. The common null polymorphism of the human GSTT1 gene has been studied extensively, but little is known about the functional consequences of GSTT1 single nucleotide polymorphisms (SNPs). We have examined the effects of two GSTT1 SNPs that alter amino acid residues in the dimer interface of the GST T1-1 protein. The variant proteins were expressed in an *E. coli* strain in which formation of mutagenic glutathione conjugates leads to lacZ reversion mutations. We also measured the activities of the variant enzymes with several substrates, including Michaelis-Menten kinetic parameters for the characteristic GST Theta substrate EPNP (1,2-epoxy-3-(p-nitrophenoxy)propane). We used circular dichroism spectroscopy to measure protein thermal denaturation profiles. Variant T104P, which has been reported as inactive, showed weak but detectable activity with each substrate. Variant R76S had higher specific activities than the wild-type enzyme with some substrates, but was expressed at lower levels and showed much-reduced thermal stability. The results are interpreted in the context of the three-dimensional structure of human GST T1-1.

17

Analyzing the Relationship between Radiation-Induced Phospho-Protein Signaling and Surrogate Cancer Endpoints Using Novel Flow-Based Assays. Whalen MK[1], Sridharan DM[1], Wilson W[1], Chapell L[2], Cucinotta FA[2], Pluth JM[1]. [1]Lawrence Berkeley National Laboratory, Berkeley, CA, United States, [2]Lyndon B. Johnson Space Center, Houston, TX, United States.

Radiation exposure induces an immediate signaling resulting in cell cycle blocks, a repair cascade, and depending on the dose and cell-type apoptosis or senescence. Studies have shown that radiation can both induce and promote carcinogenesis. Detailing phospho-protein signaling kinetics in response to radiation can offer a window into a cells response to these exposures. Some characteristic cellular changes linked to the carcinogenic process include genetic instability, telomere length changes and aberrant cellular signaling. Identifying the relationship(s) between early and late radiation-induced cell signaling, inductions in surrogate cancer endpoints, and radiation quality exposure will provide a better understanding of the mechanisms involved in cancer formation following exposure. We have initiated studies to determine critical phosphorylation signaling at early and late time points as well as surrogate cancer endpoints of telomere length changes and genomic stability as measured by centrosome amplification. Breast epithelial and fibroblast cells from the same individual will be used to understand differences in responses due unique to cell-type. Radiation quality dependent effects on phospho-protein profiles that differ dependent on cell type have been noted. Persistent signaling has also been observed in cells showing centrosome abnormalities. A detailed analysis of results to date compiling how these endpoints compare in these normal primary lines will be presented.

18

Lethal Mutagenesis of HIV and Cancer Cells by Nucleoside Analogs. Fox EJ[1], Shen JC[1], Prindle MJ[1], Harris KS[4], Mullins JI[3], Essigmann JM[5], Loeb LA[1,2]. [1]Department of Pathology, University of Washington, Seattle, WA, United States, [2]Department of Biochemistry, University of Washington, Seattle, WA, United States, [3]Department of Microbiology, University of Washington, Seattle, WA, United States, [4]Koronis Pharmaceuticals, Seattle, WA, United States, [5]Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA, United States.

The evolution of cancer and RNA viruses is characterized by the rapid accumulation of mutations, facilitating phenotypic plasticity and environmental adaptation. The mutation rates of riboviruses and retroviruses, for example, range between 10^{-3} and 10^{-5} nucleotide substitutions per replication. We have demonstrated that serial passage of virus from HIV-infected tissue cultures, in the presence of mutagenic nucleosides, results in mutation accumulation, leading to error catastrophe and viral extinction. Phase II clinical studies have shown that the administration of a cytidine analog, KP-1461, to individuals infected with HIV results in an increase in viral mutations. While the mutation frequency in normal human cells is approximately 10^{-9}, the mutation frequency in malignant cells, in the absence of prior chemotherapy, is approximately 10^{-6}. Both the random and clonal mutations found in human tumors are predominantly single nucleotide substitutions, suggesting that mutations are introduced at the level of DNA polymerization. We hypothesize that treatment of cancer cells with mutagenic nucleoside analogs will therefore also lead to a progressive accumulation of mutations until the error threshold for viability is exceeded and the tumor ablated. We have serially passaged mismatch repair-proficient and -deficient colon cancer cells in the presence of 36 different mutagenic deoxynucleoside analogs and established new protocols for measuring both mis-incorporation and repair activities during DNA synthesis in cells.

19

Role of the Circadian Clock in UV-Induced Skin Carcinogenesis. Gaddameedhi S, Selby CP, Sancar A. University of North Carolina, Chapel Hill, NC, United States.

Introduction: Skin cancer is the most common form of cancer in the United States. The main cause of this cancer is the dipyrimidine photoproducts induced in epidermal DNA by the ultraviolet (UV) component of sunlight. In mice and humans, UV photoproducts are eliminated from DNA by nucleotide excision repair (NER). Here, we describe that a rate-limiting subunit of the excision repair system, XPA, is controlled by the circadian clock in the mouse skin. As a consequence, NER exhibits daily rhythmicity in skin. The overall goal of this study is to determine whether UV exposure at certain times of the day is more likely to cause skin cancer. Methods: Skin carcinogenesis was measured in SKH-1 hairless mice that were irradiated with UV light from a sun lamp either at 4 AM or 4 PM. Measurements of protein levels, NER capacity and replication were made in control mice. Results: Our results suggest that the XPA level and the capacity to repair UV-induced DNA damage are maximal in the evening and minimal in the morning. More importantly, we found that mice exposed to UV radiation at 4 am when repair is at its minimum are more prone to skin cancer than mice exposed to UV radiation at 4 pm when repair is at its maximum. At 25 weeks, AM treated mice had two-fold more carcinomas than PM treated mice. Discussion: Our studies establish a rationale for chrono-photobiological response and suggest that timing may reduce the risk of exposure to sunlight and tanning beds.

Poster Abstracts

P1

DNA Damage-Induced Regulation of Base Excision Repair by Dynamic Localization. Bauer NC[1], Corbett AH[1,2], Doetsch PW[1,2]. [1]Emory University School of Medicine, Atlanta, GA, United States, [2]Winship Cancer Institute, Atlanta, GA, United States.

The highly-conserved base excision repair (BER) pathway is responsible for repairing the most common forms of DNA damage: oxidation, deamination, and hydrolysis of bases. Although the biochemical mechanism of BER is well characterized, few studies have addressed how this critical cellular pathway is regulated. Previous studies of a *Saccharomyces cerevisiae* BER N-glycosylase Ntg1 have revealed that dynamic localization to the nucleus and mitochondria in response to localized DNA damage is an important component of its regulation. To establish the generality of this mechanism, we are investigating the localization of uracil-DNA glycosylase (Ung1), the enzyme responsible for removing uracil (deaminated cytosine), which also localizes to both the nucleus and mitochondria. Cells expressing Ung1-GFP were treated with the deamination DNA damage agent sodium bisulfite and imaged by confocal fluorescence microscopy to assess the localization of Ung1-GFP. Cells (WT, 2μ UNG1-GFP, and ung1Δ) similarly treated were also analyzed for nuclear mutation frequency. Mutation frequency assays demonstrate that overexpressed Ung1 sensitizes cells to bisulfite-induced mutagenesis, while ung1Δ results in elevated mutagenesis without increasing sensitivity to bisulfite. This suggests that abasic sites are the primary cause of deamination-induced mutagenesis and cytotoxicity. Preliminary results from the localization studies indicate that dynamic localization in response to DNA damage occurs for Ung1, but more work is needed. A novel quantitative subcellular compartmentalization analysis (Q-SCAn) technique is being developed which will provide more quantitative results for localization studies than our current approach. This project was supported by NIH grants ES011163, GM058728, and GM008367.

P2

8-Oxoguanine, Transcriptional Mutagenesis, and Oncogenic Mutations in Mammalian Cells. Morreall J[1], Saxowsky T[2], Doetsch P[1]. [1]Emory University, Atlanta, GA, United States, [2]Pacific Lutheran University, Tacoma, WA, United States.

DNA damage occurs continuously in all cells. Transcription across DNA damage can give rise to mutant transcripts, a process called transcriptional mutagenesis (TM). The ensuing mutant proteins could contribute to tumorigenesis if the DNA damage occurs in an oncogene or tumor suppressor. 8-oxoguanine (8OG) is a common DNA lesion that causes GCàTA transversions, whose repair is primarily initiated by 8-oxoguanine glycosylase 1 (OGG1). The allelic variant S326C-OGG1 is associated with a variety of cancers, presumably due to inefficient removal of 8OG. Recent models of TM establish its role as a possible initiating event in tumorigenesis. We hypothesize that S326C-OGG1 contributes to transcriptional mutagenesis. We focused on the Ras oncogene in a DNA repair-compromised background, A model system was configured by co-transfecting S326C-OGG1 and a replication-incompetent, site-specific 8OG-bearing Ras construct into OGG1-/- mouse embryonic fibroblasts. Mutagenic transcription across this lesion gives rise to constitutively active, mutant Q61K-Ras, and thus TM can be measured by the increased level of downstream ERK phosphorylation. S326C-Ogg1 gave rise to slightly higher P-ERK signals than WT Ogg1, but the expression of S326C-Ogg1 was approximately twice as high as that of WT Ogg1. Therefore, S326C-Ogg1 is substantially less efficient in excising 8OG than WT Ogg1, indicating its substantial contribution to TM. In our *in vivo* mammalian system, we measured TM arising from allelic variants of a repair enzyme through an activated Ras phenotype, demonstrating that TM may contribute to tumorigenesis. Future work will address the kinetics of 8OG excision by S326C-OGG1 by examining the persistence of 8OG. This work was supported by NIH Grant CA120288 (to P.W.D.).

P3

Age-Dependent Accumulation of DNA Damage in Liver Tissue from APEX1 Haploinsufficient Mice. Torres-Ortiz C[1,4], Acevedo-Torres K[2], Walter CA[5], Ayala-Torres S[3], Torres-Ramos CA[1]. [1]Department of Biochemistry, University of Puerto Rico Medical Sciences Campus, San Juan, PR, United States, [2]Department of Pediatrics, University of Puerto Rico Medical Sciences Campus, San Juan, PR, United States, [3]Department of Pharmacology and Toxicology Department of Pharmacology and Toxicology, University of Puerto Rico, Medical Science Campus, San Juan, PR, United States, [4]Department of of Physiology and Biophysics, University of Puerto Rico Medical Sciences Campus, San Juan, PR, United States, [5]Department of Cellular and Structural Biology, The University of Texas Health Science Center, San Antonio, TX, United States.

Apurinic/apyrimidinic endonuclease 1 or Apex1, is a key enzyme in the process of base excision repair (BER), a process responsible for repairing the majority of the DNA lesions induced by reactive oxygen species (ROS). Repair of DNA lesions induced by ROS is important to human health since DNA damage has been linked to carcinogenesis. *In vivo* evidence suggests that DNA repair capacity declines with age and that Apex1 may be limiting in certain tissues. Studies using mice in which one copy of the APEX1 gene has been deleted (resulting in APEX1 haploinsufficiency or APEX1$^{+/-}$ mice) show increased spontaneous mutations in liver. We hypothesize that this increased mutation frequency is due to age-dependent accumulation of DNA damage. We tested this hypothesis by determining the amount of DNA damage in a 6.9kb nuclear DNA (nDNA) fragment from liver tissue in three different age groups (6, 16 and 28 months) in wild type (WT) and APEX1$^{+/-}$ mice. To detect DNA damage we applied a gene specific assay based on PCR that can detect a variety of DNA lesions such as abasic sites, strand breaks, and oxidized bases. Our results show that aging leads to increased nDNA lesions in the target nDNA sequence in both WT and Apex1$^{+/-}$ mice. Moreover, we found that the 16-month-old Apex1$^{+/-}$ mice had increased levels of nDNA damage as compared to aged matched WT mice (0.77 lesions/10kb/strand *versus* 0.37 lesions/10kb/strand, respectively). We conclude that APEX1 haploinsufficiency leads to age-dependent accumulation of nDNA damage. Sponsored by 5SC3GM08475902, 5R25GM061838-09, and G12RR03051.

P4

Identifying Increased Lung Cancer Susceptibility in Secondhand Smoke Exposed Ogg1/Myh Mice and Human Lymphocytes. Chapman AM, Yamamoto L, Schiestl R. University of California Los Angeles, Los Angeles, CA, United States.

Lung cancer is the leading cause of death from cancer in both men and women in the United States. Over exposure to environmental tobacco smoke causes lung cancer in nonsmoking individuals. We suggest that discrepancies in important DNA repair pathways may be the key to explaining lung tumorigenesis in DNA repair deficient individuals exposed to environmental tobacco smoke. In our study we focused our attention on animals deficient in base excision repair. Myh and Ogg1 are the enzymes responsible for the repair of oxidative DNA base damage. 35% of mice deficient in both Ogg1 and Myh spontaneously develop lung tumors starting after about 1 year of age. This data provides the basis of our hypothesis that cells from these mice may show elevated levels of sidestream cigarette smoke extract (CSE)- induced genetic instability since they are deficient in repair of damage produced by sidestream smoke. In our experiments we found that there was an observed significant difference in the amount of γH2AX at 3hrs between WT and Ogg -/- Myh-/- double knockout mice administered (CSE), and a significant difference in levels the amount of micro nucleius formation at 24hrs. This sSuggestsing that 1 puff/mL (CSE) caused a significant amount of double stranded breaks and chromosomal aberrations, respectively, in base excision repair deficient mice. The results of the study described above may further elucidate the mechanism of lung cancer frequency and may serve as a translational susceptibility determinate to aid in the further study of lung tumorigenesis caused by oxidative DNA damage due to secondhand smoke exposure.

P5

Elucidating DNA Repair Kinetics of Haloacetonitriles in Chinese Hamster Ovary Cells and Effect on Cell Cycle Arrest. Komaki Y, Mariñas BJ, Plewa MJ. University of Illinois at Urbana-Champaign, Urbana, IL, United States.

Drinking water disinfection by-products (DBPs) form as an unintended consequence of disinfection processes and may impose chronic risk onto the public health. Our laboratory has systematically evaluated the cytotoxicity and genotoxicity of several chemical classes of drinking water DBPs, which resulted in the largest comparative, quantitative database of DBPs of mammalian cell toxicity. DNA repair kinetics study has been done recently since not only genotoxicity induction, but also repair kinetics provides important information for the health risk evaluation associated with DBPs. In this study DNA repair kinetics was measured for three monohaloacetonitriles (HAN): chloroacetonitrile (CAN), bromoacetonitrile (BAN), and iodoacetonitrile (IAN). The single cell gel electrophoresis genotoxicity assay with Chinese hamster ovary (CHO) cells was modified to include liquid holding recovery time and was used to measure the remaining unrepaired DNA. The rank order of genotoxic potency was IAN ≈ BAN >> CAN. The concentration of each HAN was chosen to generate approximately the same level of genomic damage. Nuclei from CHO cells treated with BAN showed the lowest rate of DNA repair, followed by IAN and then CAN. These data are similar to those published for chloroacetic acid (CAA), bromoacetic acid (BAA), and iodoacetic acid (IAA) with a rank order of genotoxic potency of IAA > BAA >> CAA, and with BAA showing the lowest rate of DNA repair compared to that of CAA or IAA.

P6

The Common MUTYH Variant Q324H Has Reduced OG:A Lesion Repair in a GFP-Based Assay in Mammalian Cells. Raetz AG[1], Xie Y[1,2], Chang C[1]. [1]University of California, Davis, Davis, CA, United States, [2]University of Manitoba, Winnipeg, MB, Canada.

Biallelic germline mutations in the base excision repair enzyme gene MUTYH lead to multiple colorectal adenomas and carcinomas. MUTYH removes adenine misincorporated opposite 8-oxoguanine (OG), a common form of oxidative DNA damage that leads to G:C to T:A transversion mutations. Although biochemical assays show that many MUTYH variants have reduced DNA binding and catalytic activity, the repair of OG:A mispairs is difficult to determine in mammalian cells due to a lack of appropriate assays. In this study we developed a novel fluorescent-based assay of OG:A repair in MUTYH-/- mouse embryonic fibroblasts to study the repair capacity of human MUTYH variants. We found that OG:A repair of cancer-associated variants G382D and Y165C was significantly lower than in cells expressing the human wild-type MUTYH. We found overexpression of G382D, but not Y165C, compensated for this reduced repair. The common polymorphism Q324H, found in diverse human populations at an allele frequency of 20-49%, has been recently associated with increased cancer risk. OG:A repair in MEFs expressing Q324H was significantly lower *versus* wild-type controls when mRNA expression was equalized. We speculate that those carrying Q324H together with a cancer-associated MUTYH mutation may be at increased risk for colorectal cancer, and this may be a possible factor in the borderline increased risk of those who are currently considered monoallelic carriers. This assay will be a helpful tool to study the DNA repair capacity of MUTYH variants.

P7

A Role for DinB in Protecting *Escherichia coli* from the Lethal Effects of UV and Ionizing Radiation. Lee M-CW[1], Franco M[1], Vargas DM[1], Hudman DA[2], Fowler RG[1], Sargentini NJ[2]. [1]San Jose State University, San Jose, CA, United States, [2]AT Still University Kirksville College of Osteopathic Medicine, Kirksville, MO, United States.

We investigated the involvement of DinB (PolIV) in one or more of the several DNA repair and tolerance systems that protect *Escherichia coli* from the lethal effects of radiation. DinB, along with UmuDC (PolV), is one of the two *E. coli* Y-family translesion synthesis (TLS) DNA polymerases and both are induced as part of the SOS response. A null ΔdinB allele sensitized *E. coli* cells to both UV and X-radiation. Additional UV studies comparing the ΔdinB and wild-type strains showed that ΔdinB was most sensitive (160-fold) in early to mid-logarithmic phase and less sensitive (2-fold) in late log or stationary phase. The sensitizing effect of ΔdinB was completely dependent upon the presence of UmuDC because ΔumuDC and ΔdinBΔumuDC strains demonstrated very similar sensitivities to UV killing from early log phase to early stationary growth. The UV sensitivities of the ΔumuDC and ΔdinBΔumuDC strains were significantly greater than for the ΔdinB strain, suggesting that DinB facilitates a role of UmuDC in providing UV resistance. The ΔdinB mutation partially sensitized (5-fold) uvrA and uvrB strains to UV, but didn't sensitize recA cells, which suggest some general role for DinB in nucleotide excision repair (NER) and recombinational repair as well as presumably TLS. Based upon earlier studies that indicated that DinB can slow down the replication fork, we suggest that DinB, together with UmuDC, reduces fork speed which allows more time for radiation-induced lesions to be removed or avoided before they become lethal events.

P8

Role of Poly(ADP-ribose) Polymerase-1 in Sulfur Mustard Intoxication. Debiak M, Lex K, Lutz G, Burkle A. Molecular Toxicology Group, University of Konstanz, Konstanz, Germany.

Sulfur mustard (2,2'-dichlorodiethylsulfide; SM) is a highly toxic and mutagenic warfare agent classified as a weapon of mass destruction. As soon as SM was first used as a warfare agent, research aimed at the development of an effective antidote was launched. Early studies with first-generation inhibitors of poly(ADP-ribose) polymerases (PARP) have revealed promising therapeutic potential in SM skin injury, but the underlying mechanism remains elusive. The current renaissance of PARP-1 inhibitors in cancer chemotherapy has revived the discussion on their use for treatment of SM injury. Thus based on model substances that lack military application, *i.e.* bis(2-chloroethyl) methylamine (HN2) and 2-chloroethyl ethyl sulfide (CEES), we established a comprehensive study aiming the explanation of the role of PARP-1 in SM pathology. We have recently demonstrated that PARP-1 becomes rapidly activated in living human keratinocytes after treatment with either SM analog. The maximal PARP-1 activity was observed 5 minutes after treatment with 3 mM CEES or 100 µM HN2. To our knowledge this is the first demonstration of PARP-1 activation after treatment with mustards in in the context of live cells. Currently we are addressing the question how PARP-1 becomes activated upon treatment with mustards. PARP-1 is a first-line protein involved in the cellular response to DNA strand breaks. However, mustards do not directly induce large numbers of these lesions. Interestingly, we were also not able to detect any increased ROS level at the time of PARP-1 activation. Currently we are investigating the possible involvement of DNA repair intermediates in this process.

P9

The Role of mRNA Decay in the p53 Transcriptional Response. Melanson BD[1,2], Bose R[1,2], Hamill JD[1], Marcellus K[1,2], Pan EF[1,2], McKay BC[1,2]. [1]Ottawa Hospital Research Institute, Ottawa, ON, Canada, [2]University of Ottawa, Ottawa, ON, Canada.

The p53 tumor suppressor is a DNA damage responsive sequence-specific transcriptional activator that must be held in check to permit cell growth, viability and development. A variety of negative feedback loops exist to regulate p53 expression and activity. However, very little is known about how readily the p53 transcriptional response attenuates following transient activation of p53. Here we used a temperature sensitive variant of p53 and oligonucleotide microarrays to monitor gene expression during and following reversible p53 activation. Incubation of cells at the permissive temperature led to the increased expression of many known p53-responsive transcripts. The expression of most of these target mRNAs decreased rapidly when cells were returned to the restrictive temperature, consistent with accelerated mRNA decay. Representative 3'UTRs derived from target genes were cloned into an EGFP reporter gene to yield heterologous 3'UTR transcripts under tetracycline-regulated control. The 3'UTRs derived from unstable p53-induced mRNAs led to accelerated mRNA decay while 3'UTRs from stable transcripts did not. Sequence analysis indicates that the unstable 3'UTRs tend to be longer and rich in uridine residues compared to the stable group. This yielded a higher proportion of U-, AU- and GU-rich sequences that have been associated with mRNA decay in other model systems. We propose that the p53 transcriptional response has evolved with primarily short-lived target transcripts to afford the requisite ability of the p53 response to turn on and off rapidly under appropriate physiological conditions.

P10

Nuclear Nonhomologous End Joining Proteins Directly Impact Mitochondrial Double-Strand Break Repair in *Saccharomyces cerevisiae*. Kalifa L[1,3], Quintana DF[1], Schiraldi LK[1,2], Coles G[2], Sia RA[2], Sia EA[1]. [1]University of Rochester, Department of Biology, Rochester, NY, United States, [2]The College at Brockport, State University of New York, Department of Biological Sciences, Brockport, NY, United States, [3]The Virginia Harris Cockrell Cancer Research Center at The University of Texas MD Anderson Cancer Center, Science Park, Department of Molecular Carcinogenesis, Smithville, TX, United States.

A functional mitochondrial genome is vitally important for normal cellular function. Mitochondrial DNA (mtDNA) deletions have been associated with sporatic and inherited mitochondrial diseases, as well as neurodegenerative diseases, age-related disorders, and the aging process itself. Aging individuals often accumulate a 4977 bp mtDNA deletion that is flanked by short directly repeated sequences. Moreover, two-thirds of identified mitochondrial deletions are flanked by identical or nearly identical repetitive sequences suggesting that mitochondrial deletions often occur through direct repeat-mediated deletion (DRMD) events, however the mechanisms by which these deletions arise is unknown. Despite the evidence for these events in a variety of eukaryotic organisms, there have been no proteins identified which function in this pathway. To examine how DRMDs arise, we have developed a system for monitoring these types of events in the nuclear and mitochondrial genomes of *Saccharomyces cerevisiae*. These reporters allow us to make direct comparisons between the rates of DRMD events in both compartments under identical growth conditions. Furthermore, the mitochondrial reporter contains a unique KpnI restriction endonuclease recognition site. We have expressed KpnI fused to a mitochondrial localization signal, allowing the induction of a specific mitochondrial double-strand break. Analysis of proteins important for nuclear double-strand break repair suggests that some of these proteins are also involved in mitochondrial DRMDs. Here we report that mitochondrial DRMDs are highly dependent on the MRX (Mre11p, Rad50p, Xrs2p) and Ku (Ku70p, Ku80p) complexes. Deletion of these genes specifically affects the rate of mitochondrial DRMDs in yeast, while not effecting general mtDNA maintenance.

P11

The XLF C-Terminal Region Is Required for DNA Binding and Interaction with Ku70/80 *In Vitro* but Not for Repair of Double-Strand Breaks *In Vivo*. Mahaney BL, Yu Y, Lees-Miller SP. University of Calgary, Calgary, AB, Canada.

The most detrimental DNA lesions in the cell are DNA double-strand breaks (DSBs) and a common environmental source of these lesions is ionizing radiation. In mammalian cells, the non-homologous end-joining pathway (NHEJ) is crucial for DSB repair. XLF, the most recently discovered member of the NHEJ pathway, is thought to enhance the end-joining activity of the XRCC4-DNA ligase IV complex. To better understand how XLF acts to stimulate end-joining we have undertaken a structure-function analysis of XLF. We show that the C-terminal region of XLF is highly conserved and required for DNA binding in electrophoretic mobility shift assays. Interestingly, this region contains several *in vivo* phosphorylation sites that appear to regulate the ability of XLF to bind DNA and interact with the NHEJ core complex (Ku70/80, DNA-PKcs and XRCC4-DNA ligase IV) in GST-pulldowns and immunoprecipitations. A conserved phenylalanine in the C-terminal region is essential for DNA binding and interaction with Ku70/80. Surprisingly, the C-terminal region is not required for resolution of gamma-H2AX foci *in vivo*. These results suggest that XLF is able to enhance end-joining by DNA ligase IV without binding DNA or that a redundant mechanism exists to compensate for the loss of the C-terminal region of XLF.

P12

DNA Damage Processing by DNA Polymerase θ (POLQ) in Mammalian Cells. Yousefzadeh M[1,2], Wood RD[1,2]. [1]The University of Texas Graduate School of Biomedical Sciences at Houston, Houston, TX, United States, [2]Department of Molecular Carcinogenesis, MD Anderson Cancer Center, Science Park, Smithville, TX, United States.

The mammalian genome encodes 15 different DNA polymerases that function in DNA replication, DNA repair, immunological diversity, and the bypass/tolerance of DNA damage. DNA polymerase theta (POLQ) is an A-family nuclear DNA polymerase found in vertebrate genomes, with relatives in plants and invertebrates. The *in vivo* function of POLQ is unknown. Recombinant full length POLQ was previously demonstrated to possess low fidelity and bypass thymine glycols. Furthermore, POLQ has an efficient ability to bypass an abasic site *in vitro*, a property unique to this enzyme. Previous studies have shown increased levels of spontaneous micronuclei in erythrocytes were observed in mice defective in Polq. Moreover, bone marrow stromal cells (BMSCs) from mice lacking Polq are hypersensitive to ionizing radiation and the double strand break (DSB)-inducing agent bleomycin. We exposed Polq-deficient BMSCs and controls to commonly used DNA damaging agents to analyze the sensitivity of cells lacking Polq, and to investigate potential pathways in which POLQ may participate. Polq-deficient BMSCs are sensitive to DSB-inducing agents ionizing radiation and etoposide, but not mitomycin c, an interstrand crosslinking agent. In order to study the role of POLQ in human cells, a soluble fragment containing the polymerase domain was used to generate mouse monoclonal antibodies. In nuclear extracts of 293T cells, full length POLQ appears to be greater than 250 kDa, consistent with its predicted molecular size of 290 kDa. Further characterization of the newly created antibodies may aid in revealing the role of POLQ in human cells.

P13

Zidovudine (AZT)-Induced Genotoxicity in DNA Repair-Deficient Human Fibroblasts. Momot D[1,2], John K[1], Poirier MC[1], Olivero OA[1]. [1]National Cancer Institute, Bethesda, MD, United States, [2]Temple University, Philadelphia, PA, United States.

Zidovudine (AZT), a nucleoside analog used for therapy of HIV-1, becomes incorporated into DNA. To explore repair of AZT-damaged DNA we have used human fibroblasts taken from individuals with Xeroderma Pigmentosum A (XPA) and Blooms' Syndrome (BLM). XPA cells lack nucleotide excision repair (NER), and BLM cells are missing a recQ helicase gene. XPA and BLM cells were exposed to 0 or 100 μM AZT for 24 hours (h) or 6 days (d), and the Roche xCELLigence system was used to measure cell number, based on surface impedance. At 24 h of exposure to 100 μM AZT, XPA and BLM cell growth was 71% and 70% of the unexposed cells, respectively. At day 6 of exposure to 100 μM AZT, XPA and BLM cell growth was 66% and 56% of unexposed cells, respectively. Micronuclei (MN) were counted in cells stained with DAPI. Exposure of XPA cells to 0, 10, 100 and 200 μM AZT produced 0.73, 1.36, 1.03, and 0.86 % of cells with MN at 24 h, and 0.95, 1.48, 1.88, and 3.30 % of cells with MN at 7 days. Exposure of BLM cells to 0, 10, 100 and 200 μM AZT produced 5.97, 6.32, 11.09, and 14.12 % of cells with MN at 24 hr, and 4.45, 7.88, 7.54, and 9.23 % at 7 days. In contrast, normal human fibroblasts exposed to the same doses of AZT showed MN in ≤1.8 % of cells, suggesting that both XPA and BLM contribute to repair of AZT-DNA damage.

P14

DNA Mismatch Repair Responses to PAH-Induced Genotoxicity. Hoffman PD, Schalk V, Gibson WS, Kernan CP, Hays JB, Buermeyer AB. Oregon State University, Corvallis, OR, United States.

Environmentally ubiquitous polycyclic aromatic hydrocarbons (PAHs) are converted into reactive diol epoxide (DE) intermediates that conjugate with DNA, producing highly mutagenic and carcinogenic adducts. Evidence links human colorectal cancer and dietary exposure to PAHs. DNA mismatch-repair (MMR) deficiency increases risk of intestinal cancer, and an inability to respond appropriately to dietary genotoxins is a likely contributing factor. To address the hypothesis that MMR suppresses PAH-induced mutagenesis and carcinogenesis, and that exposure to PAHs would increase cancer risk in individuals with MMR deficiencies, we investigated biochemical responses to B[a]P-DNA lesions and the effect of MMR deficiency on the genotoxicity of (±) anti B[a]PDE. In electrophoretic-mobility-shift-assays using purified MutSalpha and oligonucleotides containing B[a]P-adducted G, MutSalpha bound mismatched B[a]P-adducted substrates with higher affinities than the corresponding matched substrates, and with similar affinities to mismatched substrates without lesions. In cellular studies, we confirmed that MMR contributes to the cytotoxicity of B[a]PDE using analyses of clonogenic survival. In mutation assays, B[a]PDE induced HPRT mutants at a significantly increased rate in matched MLH1-deficient *versus* -proficient cells. Our data demonstrate for the first time that MMR-proficiency impacts the mutagenicity of a model PAH, consistent with the hypothesis that MMR status would influence risk of PAH-induced carcinogenesis. On-going analyses will identify the mutational spectra in MMR-proficient *versus* –deficient cells, and address the biochemical mechanism of the MMR-dependent responses through analysis of PAH-lesion-provoked excision and processing.

P15

Mismatch Repair Responses to Alternative DNA Structures. Larson ED, Williams J, Ehrat EA, Johnson BR. Illinois State University, Normal, IL, United States.

Alternative DNA structure formation is associated with recombination and is a target for DNA repair factors, but the mechanisms are not defined. One common non-duplex DNA structure is G-quadruplex or G4 DNA. G4 DNA is four stranded and folds from genomic regions composed of repetitive guanine motifs. Sequences with G4 forming potential are widely found in recombination hot spots, suggesting important roles in the mechanisms of DNA rearrangement. While associated with recombination, the pathways for metabolism of G4 DNA are not well defined. We have found that the MutS homologs from human and *E. coli* recognize G4 DNA with high affinity. The MutS homologs are best known for initiating repair of replication errors, but G4 recognition does not appear to occur within the confines of the mismatch repair pathway. Both intra- and inter-molecular G4 structures are bound by MutS, and binding is independent of the DNA mismatch recognition domain. Mismatch repair activities are not activated upon G4 binding because MutS remains associated with G4 in the presence of ATP. Our results support a conserved ability for MutS proteins to bind G4 DNA outside of the classically defined post-replication repair pathway, suggesting a new function for the complex in the recombination or metabolism of non-duplex DNA.

P16

Two Novel Mutations on the XPG Gene Affect Two Brazilian Xeroderma Pigmentosum Patients. Menck CFM[3], Rocha CRR[3], Cabral-Neto JB[1], Sarasin A[2], Soltys DT[1]. [1]Instituto de Biofísica Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil, [2]Centre National de la Recherche Scientifique FRE2939, Institut Gustave Roussy, University Paris-Sud, Villejuif, France, [3]Department of Microbiology, Institute of Biomedical Science, University of São Paulo, São Paulo, SP, Brazil.

In this work, the genetic defect on the XPG gene was investigated in two Brazilians xeroderma pigmentosum (XP) patients, siblings and mildly affected, diagnosed as XP-G by the heterodikaryon complementation test. The cells from these patients present high UV sensitivity, typical for XP cells, but they are not sensitive to methylene blue (MB) plus light, an agent that causes oxidative stress, as observed for XP-G/CS cells. The sequence of both mRNA and genomic DNA revealed two novel missense mutations in a compound heterozygosis manner: c.83C>A (p.Ala28Asp), located in the paternal allele, and c.2904G>C (p.Trp968Cys), in the maternal allele. Analyzing the impact of the aminoacid substitutions through the internet tools SIFT and PMut predicts that p.Ala28Asp and p.Trp968Cys have a negative impact in the protein function. The impaired functions of these allelic forms of XPG were confirmed by host cell reactivation assays, where the mutated genes were not capable to complete restore the DNA repair deficiency in XP-G/CS cells in UV irradiated plasmids. However, full correction was observed in plasmids damaged by photosensitized MB. These results indicate that the phenotype of XP-G patients is caused by two missense mutations in a compound heterozygous manner, which correlates well with the mild clinical phenotype presented, and that the cells carrying these mutations exhibit different responses against genotoxic stress caused by the UV light and by the oxidative agent used. Financial Support: FAPESP (São Paulo, Brazil) and CNPq (Brasília, Brazil).

P17

Transcription Arrest by a G Quadruplex Forming-Trinucleotide Repeat Sequence from the Human c-Myb Gene. Broxson C, Beckett J, Tornaletti S. Department of Anatomy and Cell Biology University of Florida College of Medicine, Gainesville, FL, United States.

Repetitive DNA sequences with propensity to form non-canonical (non-B) DNA structures frequently correspond to fragile sites commonly found in human disease. Tanscription facilitates formation of non-canonical structures and participates in generating the instability associated with these sequences. However, little is known about how non-B structures are processed by transcription. Here we have studied the behavior of T7 RNA polymerase (T7RNAP) when encountering a G quadruplex forming-$(GGA)_4$ repeat located in the human c-myb proto-oncogene. To make direct correlations between G4 formation and effects on transcription we have started to analyze transcription on single stranded DNA substrates in which the G4 DNA structure was induced by incubation in the presence of potassium ions. Under physiological KCl concentrations, we found that T7 RNAP was arrested at two sites that mapped to the c-myb $(GGA)_4$ repeat. Consistent with G4 DNA formation, arrest was not observed in the absence of KCl or in the presence of LiCl. Furthermore, base substitutions in the c-myb repeat, expected to prevent transition to G4, also eliminated the transcription block. We show T7 RNAP arrest at the c-myb repeat in double stranded DNA under conditions mimicking the cellular concentration of biomolecules and potassium ions, suggesting that this G4 structure may represent a transcription roadblock *in vivo*. Our results support a mechanism of transcription-coupled DNA repair initiated by arrest of transcription at G4 structures.

P18

Effect of Spontaneous DNA Lesions on DNA Structural Transitions Occurring at the Nuclease Hypersensitive Site of the c-Myc Proto-Oncogene. Beckett J, Broxson C, Tornaletti S. Department of Anatomy and Cell Biology University of Florida College of Medicine, Gainesville, FL, United States.

The promoter of the c-myc proto-oncogene contains several regulatory elements that have the propensity to assume non-canonical DNA structures under physiological conditions. Among them, the nuclease hypersensitive element III_1 ($NHEIII_1$) consists of a polypurine/polypyrimidine tract that can transition from duplex to quadruplex (G4) DNA *in vivo*. This promoter element localizes near the most frequent translocation hot spot for the c-myc gene in B-cell malignancies, suggesting that the structural transitions occurring at this site may be involved in generating the fragility associated with this genomic region. We hypothesize that formation of non-canonical structures in this sequence may impair recognition and repair of DNA damage localized at or near this site, potentially leading to mutagenesis. To test this model, we have started to characterize the effect of two spontaneous DNA lesions, the abasic site and 8-oxoguanine, which are expected to form at high incidence in the $NHEIII_1$, on the structural transitions occurring at this site. We show by dymethylsulfate footprinting and transcription assays that under physiological concentrations of potassium ions the $NHEIII_1$ folds into two G4 structures, the chair and the basket. We find that the presence of a single abasic site or a single 8-oxoguanine within the $NHEIII_1$ affects the type of G4 structure formed and in some cases, formation of the structure itself. These effects are dependent on the location of the lesion within the G repeat.

P19

G4 DNA Structures and Genome Evolvability of the Human Pathogen *Pseudomonas aeruginosa*. Tornaletti S[1], Brocchieri L[2]. [1]Department of Anatomy and Cell Biology, University of Florida College of Medicine, Gainesville, FL, United States, [2]Department of Molecular Genetics and Microbiology, University of Florida College of Medicine, Gainesville, FL, United States.

P. aeruginosa is an opportunistic pathogen of growing relevance for human health for its adaptability in infecting virtually any compromised human tissue and for its resistance to antibiotic treatment. Its adaptability reflects in the unusual plasticity of its genome structure and genotypic and phenotypic response to different environmental conditions, including chromosomal insertions or rearrangements, alternative morphological forms, acquisition of genetic elements conferring virulence, and frequent emergence of mutator genotypes in clinical isolates. The genome of *P. aeruginosa* is characterized by high GC content, which facilitates formation of G quadruplex (G4) DNA structures, DNA alternative secondary structure elements known to affect genome stability and gene expression regulation. The “hyper-variable” region of the *P. aeruginosa* flagellin gene is characterized by insertions/deletions and a high rate of amino acid substitution. This hyper-variable region is also characterized by an unexpectedly high rate of synonymous substitution, suggesting enhanced fragility of these sequences. Bioinformatics tools predict in this region of the flagellin gene (and not in its conserved region) formation of G4 DNA, We provide new insights into the role of DNA topography in *P. aeruginosa* adaptation strategies, characterizing with a comparative-genomics study of several *P. aeruginosa* strains, the relation of hot-spots of mutation with the sequence potential for formation of G4 structures. Furthermore, we experimentally verify G4 DNA formation by chemical footprinting and transcription arrest assays.

P20

Removal of Oxidized Bases from Quadruplex DNA and Telomeric DNA Sequences by the Mammalian DNA Glycosylases. Zhou J[1], Liu M[1], Fleming AM[2], Burrows CJ[2], Wallace SS[1]. [1]University of Vermont, Burlington, VT, United States, [2]University of Utah, Salt Lake City, UT, United States.

The telomeric DNA of vertebrates consists of tandem repeats of $d(TTAGGG)_n$, which can form quadruplex DNA structures *in vitro* and likely *in vivo*. Despite the fact that the G-rich telomeric DNA is susceptible to oxidation, few biochemical studies of base excision repair in telomeric DNA have been done. Here we show that telomeric DNA with thymine glycol (Tg), 8-oxoguanine (8-oxoG), guanidinohydantoin (Gh) or spiroiminodihydantoin (Sp) can form quadruplex DNA structures. We have tested the base excision activities of five mammalian DNA glycosylases (NEIL1, NEIL2, MmuNeil3, NTH1 and OGG1) on these lesion-containing quadruplex DNA substrates. Among these glycosylases, only MmuNeil3 showed excision activity on Tg in quadruplex DNA, and none of the glycosylases had activity on 8-oxoG in quadruplex DNA. Surprisingly, MmuNeil3 exhibited a strong preference for Tg in the telomeric sequence context compared to the non-telomeric sequence context. We also found that Sp and Gh lesions in quadruplex DNA were good substrates for MmuNeil3. These data suggest that one role for Neil3 is to repair oxidized DNA bases in telomeres and function in telomere maintenance *in vivo*. Acknowledgement: This work was supported by NIH Grant P01 CA098993 awarded by National Cancer Institute.

P21

DNA Replication and Intra-S Checkpoint Function in UV-Damaged Human Melanocytes. Kaufmann W, Zhou Y, Smith-Roe S, McNulty J, Simpson D, Chastain P, Gaddameedhi S, Sancar A, Sproul C, Cordeiro-Stone M. University of North Carolina at Chapel Hill, Chapel Hill, NC, United States.

Solar ultraviolet radiation is an established human skin carcinogen, inducing squamous and basal cell carcinoma’s and melanoma. We are investigating the mechanisms of solar radiation-induced melanomagenesis. The UV wavelengths that damage human skin (UVB/UVA) produce DNA photodimers (CPD’s and 6,4-PP’s) and oxidative DNA damage. Three independent strains of normal human skin melanocytes (NHM’s) were shown to repair UVC-induced CPD’s and 6,4-PP’s efficiently at rates similar to skin fibroblasts. Eighty percent of 6,4-PP’s were removed from fibroblast and melanocyte DNA within 3 h after irradiation. Irradiation with UVC inhibited DNA replication in NHM’s. Low fluences of UV produced a selective inhibition of replicon initiation with higher fluences also inhibiting DNA chain elongation in active replicons and increasing the production of abnormally small nascent intermediates. NHM’s displayed an effective intra-S checkpoint response to UVC with rapid activation of Chk1, Chk2 and ATM. Depletion of Chk1 using siRNA attenuated the inhibition of DNA synthesis that was seen in NHM after low fluences of UVC. We surveyed nine melanoma cell lines for defects in intra-S checkpoint signaling and post-replication repair (PRR). Eight of the lines displayed effective nucleotide excision repair, checkpoint signaling and PRR. One displayed defects in nucleotide excision repair and PRR. The analysis of melanoma lines suggests that the essential functions of intra-S checkpoint genes (ATR, Chk1, Timeless) are preserved during melanomagenesis. This analysis also demonstrates that lessons about UV photobiology derived from study of human skin fibroblasts are replicated in human skin melanocytes. Supported by PHS grants ES10126, ES015856, ES014635.

P22

Contributions of the Replication Fork Protection Complex, Timeless-Tipin, to Multiple Genome Maintenance Functions. Smith-Roe SL, Patel SS, Zhou YC, Simpson DA, Kaiser-Rogers KA, Cordeiro-Stone M, Kaufmann WK. University of North Carolina at Chapel Hill, Chapel Hill, NC, United States.

Replication forks constantly encounter challenges - from chemical modifications of DNA to intrinsic characteristics of DNA sequence and metabolism - yet genomes are replicated with tremendous accuracy. The Timeless-Tipin complex interacts with components of the replication machinery (MCM2-7 helicase, replicative polymerases), checkpoint proteins (RPA, Claspin), and cohesin ring subunits, indicating a role for Timeless-Tipin in replication-dependent genome maintenance. Although we and others have shown that Timeless, Tipin, and Claspin work together to mediate ATR-dependent activation of Chk1 kinase in the intra-S checkpoint response to DNA damage, these proteins have different contributions to DNA replication, as do ATR and Chk1, in normal human fibroblasts (NHF). Depletion of Timeless, Tipin, and Chk1 results in cessation of DNA replication, which was not as apparent with depletion of Claspin, and not detected with depletion of ATR. Reduced incorporation of BrdU in Timeless- or Tipin-depleted NHF was accompanied by ATR- and Claspin-dependent activation of Chk1; however, co-targeting of Timeless and ATR did not restore normal BrdU incorporation, indicating a role for Timeless in DNA replication upstream of ATR. Timeless, Tipin, and Claspin contributed to sister chromatid cohesion (SCC) independently of Chk1, and Timeless had a Tipin-independent contribution to SCC. Depletion of Timeless also had a greater effect on chromosome instability than depletion of Tipin, Claspin, or Chk1, possibly due to impaired cohesion in Timeless-depleted NHF. ATR-depleted NHF failed to activate the DNA damage response despite considerable chromosome instability. Presently, we are characterizing a doxycycline-inducible system in NHF for structure-function studies of Timeless and Tipin.

P23

Role of DNA Polymerase η in Mitochondrial Mutagenesis of *Saccharomyces cerevisiae*. Chatterjee N, Pabla R, Siede W. University of North Texas Health Science Center, Fort Worth, TX, United States.

Introduction: DNA polymerase η functions as a eukaryotic translesion polymerase that can bypass the most frequent types of UV damage in an error-free mode. Since nucleotide excision repair is absent in mitochondria, the presence of efficient bypass mechanisms could easily be rationalized. In *Saccharomyces cerevisiae*, there is indeed evidence that Pol ζ (Rev3/Rev7) plays a role in mitochondrial UV mutagenesis but nothing is known about Pol η. Methods: We studied localization of Pol η (Rad30) microscopically and biochemically, using GFP- and Myc-fusions. Mitochondrial mutagenesis was determined using various systems: petite induction, base substitution leading to erythromycin resistance, arg4 frameshift reversion. Results: We show that Pol η localizes to nucleus and mitochondria. Curiously, strong overexpression results in exclusion from mitochondria. We also detected its influence on mitochondrial base substitution and frame shift mutation following UV radiation: as in the nucleus, its deletion enhances UV mutability. The same had been found for base substitutions in Pol ζ mutants (1), a surprising result given its error-prone function in the nucleus. We confirmed this result and showed near-epistatic interaction with rad30 for UV-induced base substitution. Discussion: Yeast Pol η plays an error-reducing role in certain modes of mitochondrial UV mutagenesis – for base substitutions apparently by functioning in the same pathway as Pol ζ. This leaves one with the intriguing question whether polymerase γ is responsible for mutagenic events that are being avoided by the action of Pol ζ and η. (1) Kalifa, L., Sia, E.A. DNA Repair 6 (2007) 1732.

P24

Analysis of Molecular Splint Mutants of Human DNA Pol Eta and Their Effect on Polymerase Properties. Beardslee RA, McCulloch SD. North Carolina State University, Raleigh, NC, United States.

DNA polymerase η (pol η) is responsible for the bypass of both cyclobutane pyrimidine dimers (CPDs) and 8-oxoguanine (oxoG) during DNA replication. Both are ubiquitous; the former is produced by exposure to UV radiation, while the latter is generated by reactive oxygen species (ROS). It follows that pol η is indispensable to successful DNA replication and organism survival. It has been reported that amino acids 316-324 appear important to correctly align the template strand with the catalytic core of the enzyme. We hypothesized that modification of these residues would interfere with that alignment and alter enzyme activity and fidelity. To study the effect of these mutations, we expressed the catalytic core of human pol η in *E. coli* with single amino acid substitutions at residues 316, 318, 320, 322 and 324 in addition to wild type enzyme. Overexpressed protein was purified by chromatography using HiTrap™ Chelating HP (GE) with subsequent application of pol η rich fractions to Mono S™ (GE). Purified protein fractions and DNA oligomers synthesized with both CPD and oxoG lesions were used in *in vitro* assays to evaluate polymerase properties during replication of undamaged and damaged DNA. We find that certain mutations alter the activity and/or fidelity of pol η compared to wild type and propose that these results suggest that these amino acids are important for successful bypass of DNA lesions. Furthermore, we believe that the findings will contribute to an explanation for the low bypass fidelity of pol η past oxoG lesions.

P25

Mitochondrial Dynamics and Autophagy Aid in Removal of Persistent Mitochondrial DNA Damage. Bess AS, Crocker TL, Ryde IT, Meyer JN. Duke University, Durham, NC, United States.

Mitochondria lack the ability to repair helix-distorting lesions that are induced at high levels in mitochondrial DNA (mtDNA) by important environmental genotoxins and endogenous metabolites. These lesions are irreparable and persistent in the short term, but their long-term fate is unknown. We investigated the removal of photodimers in mtDNA *via* mitochondrial fusion, fission and autophagy in *Caenorhabditis elegans*. Larval fusion, fission and autophagy mutant *C. elegans* were exposed to serial UVC doses over 48 hours which results in an accumulation of mtDNA damage and measurable larval growth arrest mediated by mitochondrial dysfunction. Strains carrying mutations in autophagy and fusion genes exhibited exacerbated larval growth arrest. We concluded that these processes are required for normal recovery from mtDNA damage-induced larval growth arrest. To test directly the contribution of mitochondrial dynamics and autophagy proteins to removal of UVC-induced DNA damage, we performed RNAi knockdown of fusion, fission and autophagy genes in UVC treated adult glp-1 *C. elegans*. Knockdown of fusion genes eat-3 and fzo-1, fission genes drp-1 and fis-1 and autophagy/mitophagy genes bec-1, unc-51, and pink-1 inhibited removal of UVC-induced DNA damage, as measured by a quantitative PCR assay. These data indicate that removal of persistent mtDNA damage requires mitochondrial fusion, fission and autophagy, providing genetic evidence for a novel mtDNA damage removal pathway. Mutations in genes in these pathways exist in the human population, demonstrating the potential for important gene-environment interactions affecting mitochondrial health after genotoxin exposure.

P26

Pilot Study to Evaluate Mitochondrial DNA Damage As a Biomarker of Oxidative Stress in Smokers and Non-Smokers. Miranda MP[1], Narváez DM[1], Van Houten B[2], Groot de Restrepo H[1]. [1]Universidad de los Andes, Bogotá, Colombia, [2]Department of Pharmacology and Chemical Biology, Pittsburgh University, Pittsburgh, PA, United States.

Cigarette smoking is a public health problem that causes different systemic diseases. Since many of these pathologies are associated to a state of oxidative stress, mitochondria has been considered a better biomarker of exposure to this substance. The aim of this study was to evaluate mitochondrial DNA as a biomarker of oxidative stress in smokers and no smokers. To achieve this, nuclear DNA damage was determined using the Comet assay and mitochondrial DNA damage was determined with the long amplicon quantitative PCR in 5 smokers and 5 non-smokers. There were no statistical differences between smokers and non smokers either in mitochondrial or nuclear DNA. Also there was no correlation between nuclear and mitochondrial damage. Further studies are required using the long amplicon quantitative PCR for a nuclear gene to measure the same type of DNA damage in nuclei and mitochondria and increasing the sample size to determine if mitochondrial DNA damage is a better biomarker for oxidative stress.

P27

Human Mitochondrial DNA Polymerase γ Exhibits Potential for Bypass and Mutagenesis at UV-Induced Cyclobutane Thymine Dimers. Kasiviswanathan R[2], Gustafson M[1], Copeland W[2], Meyer J[1]. [1]Duke University, Durham, NC, United States, [2]NIEHS, Research Triangle Park, NC, United States.

Cyclobutane thymine dimers (T▫T) comprise the majority of DNA damage caused by short-wavelength ultraviolet radiation. These lesions generally block replicative DNA polymerases and are repaired by nucleotide excision repair (NER) or bypassed by translesion polymerases in the nucleus. Mitochondria lack NER, and therefore it is important to understand how the sole mitochondrial DNA polymerase, pol γ, interacts with lesions such as T▫T that are not repaired in the mitochondrial genome. We performed *in vitro* DNA polymerization assays to measure the kinetics of stalling or bypass by pol γ with a dimer-containing template. Pol γ bypassed thymine dimers, although with low relative efficiency. When bypass did occur, pol γ misincorporated a guanine residue opposite the 3' thymine only four-fold less efficiently than it correctly incorporated adenine. In the presence of all four dNTPs, pol γ extended the primer following either an adenine or a guanine. Our results suggest that T▫T most often stalls mitochondrial DNA replication, but also provide a possible mechanism for the introduction of point mutations and deletions in the mitochondrial genomes of chronically UV-exposed human skin cells.

P28

TFAM, MtDNA and Their Interactions during Aging and Calorie Restriction (CR) Diet. Picca A[1,3], Lezza AM[2], Leeuwenburgh C[1], Tornaletti S[3]. [1]Department of Aging and Geriatric Research, College of Medicine, Institute on Aging, University of Florida, Gainesville, FL, United States, [2]Department of Biochemistry and Molecular Biology "E. Quagliariello", University of Bari, Bari, Italy, [3]Department of Anatomy and Cell Biology, University of Florida, College of Medicine and UF Genetics Institute, Gainesville, FL, United States.

According to the "mitochondrial theory of aging" mitochondria are intensely involved in origin of aging through an endogenously production of oxidative stress. This theory stresses the relevance of mtDNA oxidative damage responsible for the dysfunctional respiratory complexes assembly and the accumulation of mtDNA mutations, thus initiating the progressive decrease of cellular energy availability. Calorie restriction (CR) is, so far, the only treatment able to delay or avoid the onset of some age-related features in various species. Mitochondrial transcription factor A (TFAM) is a nuclear-encoded protein that plays critical roles in multiple aspects to maintain the integrity of mitochondrial DNA: transcription, replication, nucleoid formation, damage sensing and DNA repair. Due to its close relationship to mtDNA, TFAM might be relevant also in aging. The well known age-related mtDNA oxidative modifications and the recent finding of a differential binding of the Lon protease along mtDNA following a chemically-induced oxidative stress, prompted us to test TFAM binding to mtDNA in the age-related physiological oxidative stress condition of rat brain and liver and to evaluate if the CR could affect TFAM-binding. We assayed several regions along the mtDNA molecule performing a semi-quantitative PCR analysis with the mtDNA immunoprecipitated by TFAM and we found a different, tissue-specific binding of TFAM with aging and CR treatment. Our results suggest that the differential binding of TFAM to mtDNA is probably associated with the functional relevance of the region bound, where it can perform specific functions (ie replication and transcription) with respect to the canonical maintenance of mtDNA.

P29

NAMPT: Connecting DNA Repair and Cellular Energetics. Ortiz S[1], Schamus S[2], Goellner EM[2], Sugrue K[2], Sobol RW[2], Almeida KH[1]. [1]Rhode Island College, Providence, RI, United States, [2]University of Pittsburgh Cancer Institute, Pittsburgh, PA, United States.

Nicotinamide adenine dinucleotide (NAD^+) is an essential cellular metabolite, serving as a coenzyme for oxidation/reduction reactions and as the substrate for post-translational modification proteins such as PARP1, among many others. NAMPT is the rate-limiting enzyme in the NAD^+ salvage pathway and has been implicated in numerous human disorders including diabetes, heart disease and cancer, making NAMPT an attractive drug target. One current drug being evaluated clinically is FK866, a potent and selective, inhibitor of NAMPT. However, the low bioavailability and rapid clearance exhibited by FK866 suggests alternative inhibitors may be more effective. NAMPT is a 55 kDa protein that forms a homodimer whose active site is located along the dimerization plane. NAMPT has several phosphorylation sites, one of which (Y188) projects into the dimerization plane. We investigated the phosphorylation status of residue Y188 on the activity of NAMPT by studying two phospho-mimetic mutations: Y188D representing the phosphorylated state and Y188F, representing the non-phosphorylated state. NAMPT cDNA (WT and mutants) was cloned into Gateway entry vectors, sequenced and recombined into destination vectors specific for both yeast 2-hybrid (Y2H) analysis and bacterial protein expression. Y2H analysis was used to evaluate dimer formation. Recombinant protein containing an N-terminal 6X-histidine epitope tag was purified from bacteria using a Ni-NTA resin and NAMPT activity was assessed using fluorescence spectroscopy. Together, these analyses will be instrumental in evaluating the impact of Y188 phosphorylation on NAMPT dimer formation and enzymatic activity and will provide a framework for identifying novel compounds as inhibitors of NAMPT.

P30

Ursolic Acid Improves DNA Repair Kinetics after Hydrogen Peroxide-Induced DNA Damage in Human Fibroblast. Santos RA, Oliveira PF, Munari CC, Silva MLA, Cunha WR, Tavares DC. Universidade de Franca, Franca, SP, Brazil.

Introduction: Ursolic acid (UA) is pentacyclic triterpenoid compound found in foods and medicinal herbs, in the form of aglycones or as a free acid. This compound is known for its hepatoprotective, anti-inflammatory, antimicrobial, hypoglycemic, antioxidant and anti-fertility activities. The aim of present study was to evaluate the cytotoxicity and antigenotoxicity effects of UA by DNA repair kinetics in the human fibroblast cell line (GM07492A), using the XTT and Comet assays. Methods: The Cytotoxicity was determined with concentrations ranging from 2 to 512 µM by the XTT assay. The Alkaline version of comet assay was employed to assess the DNA repair kinetics with 8, 16 and 32 µM of UA associated with hydrogen peroxide (H_2O_2; 50 µM). Cells were harvested immediately after DNA damage induction with H_2O_2 (T0), and 1h (T1) and 3h (T2) after recovery with UA. Results: The results showed that UA was cytotoxic at concentrations above 32 µM. The comet assay analysis revealed that UA did not present genotoxic activity at the non-cytotoxic tested concentrations. At T1 and T2 time points, cells treated with UA exhibited a significant reduction in tail intensity when compared to H_2O_2 treatment alone. This reduction was not dose dependent and did not reach the levels exhibited by the negative control. Discussion: The antioxidant properties of UA may be related to the improvement of DNA repair kinetics after H_2O_2 treatment. The next step will be to investigate the molecular pathways related to these effects in response to UA treatment.

P31

***In Utero* DNA Repair: Current Understanding and Potential Application in Risk Assessment.** Pachkowski B[1], Guyton K[2], Sonawane B[2]. [1]ORISE, U.S. Environmental Protection Agency, Washington, DC, United States, [2]U.S. Environmental Protection Agency, Washington, DC, United States.

Exposure to genotoxic chemicals during *in utero* development may ultimately lead to outcomes such as cancer, malformations, or functional deficits. As a mechanism that can limit the impact of genotoxicants in adults, DNA repair may also be an important factor that determines the outcome of the conceptus. We reviewed the literature to examine the current understanding of DNA repair during *in utero* mammalian development. Most data have been derived from studies in rodent models focusing on DNA repair gene expression, which can vary according to developmental stages, tissues, and DNA repair pathways. Gene expression information is limited for humans but is suggestive that the major repair pathways exist during *in utero* development. Due to the complexities of DNA repair and its regulation by other pathways, available gene expression data may be limited for clarifying the role of DNA repair as a mechanism controlling the response to *in utero* exposures to genotoxicants. While not a comprehensive dataset, functional studies assessing *in utero* DNA repair capacity do demonstrate the variable ability of fetal tissue to repair DNA damage. Some of the major data gaps identified are a lack of information for certain DNA repair pathways, windows of exposure, and for specific tissues, particularly within the context of human development. We conclude that data on *in utero* DNA damage and repair for quantitative health risk assessment of genotoxic chemicals are inadequate. Disclaimer: The views expressed are those of the authors and do not necessarily represent the views and/or policies of the U.S. EPA.

P32

Progressive Neuropathy in 17-Year Old Girl with Photosensitivity. Manchester D[1], Falik-Zaccai T[2], Spivak G[3]. [1]Children's Hospital Denver, UCDenver, Aurora, CO, United States, [2]Western Galilee Hospital, Nsharia, Rappaport Faculty of Medicine, Technion, Haifa, Israel, [3]Stanford University, Menlo Park, CA, United States.

Cockayne syndrome (CS) is a genetically heterogeneous, variably expressed disorder of transcription-coupled DNA repair. Phenotype-genotype correlations are not completely understood. We present a 17-year old girl with a mild form, CS type III. Sequences of ERCC6, ERCC8, and XPA cDNAs were normal. Her cells were sensitive to the oxidant, menadione and to UVC in a colony forming assay, consistent with a CS phenotype. The patient presented at 12 months with microcephaly, failure to thrive and developmental delay. Congenital infection had previously been addressed and her karyotype was normal. Her parents recognized photosensitivity as an infant; she has become more photosensitive with age. On initial examination, she was undergrown, undernourished, very microcephalic, and hypertonic. She was not sitting, but would smile and follow. Her ophthalmologic exam was normal. A cranial CT scan at 12 years was negative for calcifications. She developed tremors at 4 years, titubation at 14 years and is now starting to have choreiform movements. She has never had seizures. Her eyes are examined yearly; she does not have cataracts or retinal dyplasia. Currently, she is in high school, functioning at a second grade level. She suffers from anxiety, exhibits progressive memory loss and has psychotic episodes. This patient provides a model of progressive neuropathy that may contribute to a better understanding of the neurobiology of aging.

P33

Repression of Human Activation Induced Cytidine Deaminase by miR-93 and miR-155. Borchert GM, Holton NW, Larson ED. Illinois State University, Normal, IL, United States.

Activation Induced cytidine Deaminase (AID) targets the immunoglobulin genes of B cells, where its activity leads to mutations that result in antibody gene diversification and improved immunity. While essential for proper immune responses, misregulated AID activity can promote molecular mutagenesis by introducing genomic rearrangements and base damage that cause cancer. Importantly, AID expression has recently been observed in several epithelial cell cancers, suggesting it may contribute to mutator phenotypes in non-B cell carcinomas. Aberrant AID expression is likely repressed by specific molecular regulators, but these are not fully defined. Our analysis of the human AID mRNA transcript has revealed an extensive and uncharacterized 3' UTR encoding both miR-93 and miR-155 microRNA recognition sites, and both miR-93 and miR-155 are present in the Burkitt's lymphoma, Ramos, and in the AID-expressing human breast carcinoma MCF-7. Co-transfection of AID-negative HEK 293 cells with AID-3'UTR luciferase reporter fusions and either miR-93 or miR-155 expression constructs resulted in strong translational repression of luciferase. Consistent with a capacity for AID translational regulation, we also demonstrate that over-expression of miR-93 and miR-155 in MCF-7 cells significantly reduces AID protein levels whereas depletion of endogenous miR-93 or miR-155 conversely result in increased AID protein expression. Our results support a model whereby miR-93 and miR-155 function to reduce the potential for AID-induced genome instability and mutagenesis. Considering that the intronic miR-93 is expressed along with the MCM7 replication gene, this microRNA may act as a ubiquitous genome sentry that prevents mutagenesis through the repression of errant AID translation.

P34

Aberrant Expression Profiles of MicroRNAs in the Lung of Mice Exposed to Benzo(a)pyrene. Liang G[1], Bai Y[2], Li X[1], Zhang Y[1], Wang X[1], Yin L[1], Pu Y[1]. [1]Key Laboratory of Environmental Medicine Engineering, Ministry of Education, School of Public Health, Southeast University, Nanjing, China, [2]State Key Laboratory of Bioelectronics, Southeast University, Nanjing, China.

Introduction: MicroRNAs (miRNAs) have been implicated to play important roles in the development of cancer. Many studies have shown aberrant expression profiles in cancer tissue compared with healthy tissue, however, little is known regarding the response of miRNAs expression following to environmental carcinogens exposure. We used benzo(a)pyrene [B(a)P], a widely distributed environmental carcinogens in foods and air, to investigate the changes in miRNAs expression in lung tissue of mice and analyze the potential mediate mechanism of miRNAs. Methods: Mice were treated with 5μg/kg B(a)P (twice a week) for 8 weeks by intragastrical administration following another 8 weeks without exposure. The total RNA was isolated from lung tissues. The miRNAs expression profiles were analyzed by SOLiD deep sequencing. Results: 439 miRNAs were identified in the lung tissue of exposure mice, and 279 miRNAs were identified in the control. A total of 74 miRNAs were significantly down-regulated or up-regulated at lease 2-fold and 18 miRNAs were changed more than 4-fold in B(a)P exposed samples *versus* controls. The most remarkably changed miRNAs belonged to the families of mir-1, miR-7, miR-20, miR-21, miR-106, miR-122, miR-128, miR-130, miR-133, miR-181, miR-205, miR-296, miR-363, miR-365, miR-489. Functional and pathway analysis of the predicted targets of the some altered miRNA indicated that B(a)P exposure potentially alters signaling pathways associated with proliferation, apoptosis and carcinogenesis. Discussion: B(a)P exposure changed the miRNA expression profile in lung tissue of mice, which may lead to the modification of cellar biological process and the initiation of tumorigenic disease.

P35

Integration of MicroRNA and MRNA in Primary Mouse Hepatocytes to Identify Mechanisms of Toxicity for True Genotoxins, False Positive Genotoxins and Non-Genotoxins. Lizarraga D[1,2], Rieswijk L[1,2], Brauers K[1], Kleinjans JC[1,2], van Delft JHM[1,2]. [1]Department of Toxicogenomics, Maastricht University, Maastricht, Limburg, Netherlands, [2]Netherlands Toxicogenomic Center, Maastricht, Limburg, Netherlands.

Chemicals with carcinogenic and mutagenic potential such as genotoxins (GTX) are associated with increased incidence of several human cancers. Toxicological studies designed to assess safety of compounds for humans, frequently rely on the use of *in vitro* systems. Toxicogenomics, *i.e.* mRNA profiling, has been proven successful in prediction of toxicity and understanding mechanisms. Recently microRNA are discovered as negative regulators of mRNAs, thus integrating mRNA and microRNA response patterns can add an extra dimension to our understanding of toxic mechanisms. The aim of this study is to obtain microRNA-mRNA complexes as molecular signatures to increase our understanding of cellular mechanisms induced by genotoxins in sandwich-cultured primary mouse hepatocytes from male C57BL/6 mice. Primary mouse hepatocytes were treated for 24 and 48h with three true-GTX, two false-positive-GTX and 4 non-GTX compounds. MicroRNA and mRNA expression modifications were analyzed by Exiqon and Affymetrix platforms respectively. Phosphorylation of H2AX, as biomarker for DNA damage, was measured by immunostainings. In this study the comparison between true-GTX, false-positive-GTX and non-GTX compounds was done by combining the phenotypic DNA damage measurement, microRNA and mRNA data. MicroRNA-mRNA complexes were identified as a signature of genotoxicity. In particular, some of the microRNA-mRNA signatures were detected in specific genotoxic-responsive pathways, such as apoptosis/DNA damage response, cell cycle arrest and DNA damage repair using Metacore. Eventually, the microRNA-mRNA signatures detected in this study may contribute to improve accuracy in chemical safety predictions and to better distinguish between true-GXT and false-positive-GTX compounds.

P36

Withdrawn.

P37

Characterization of the Aneugenic Response. Rubitski E, Spellman R, Heard P, Schuler M. Pfizer, Groton, CT, United States.

Aneuploidy is a serious health problem and the mechanisms involved in aneuploidy induction are poorly understood. Many aneugenic chemicals have been identified in the *in vitro* micronucleus assay, however further classification of their mechanism of action has been lacking. The aim of the presented work is to evaluate the mechanism of action for three known aneugens; colchicine, cytochalasin B (CYB) and phenolphthalein (PHT). We have performed image based assays using CHO cells, and flow cytometric assays using TK6 cells to determine the potential cellular targets and cellular activities affected by these aneugens. A 24-27 hour direct test condition was chosen for each evaluation. The following endpoints were compared; tubulin content, actin content, centrosome enumeration, apoptosis, and mitotic inhibition. Our data indicate that centrosome amplification, changes in tubulin content and mitotic inhibition of the cell cycle are critical events that lead to the aneugenic response. These endpoints allow for a thorough evaluation of each chemical's mechanism of action and therefore, by implementing these follow up assays a more comprehensive risk management strategy for unknown aneugenic compounds can be developed.

P38

Electronic Waste Leachates Induced DNA Damage in Mice and Human Peripheral Blood Lymphocytes. Alabi OA[1,2], Li B[3], Huo X[3], Bakare AA[2]. [1]Babcock University, Ilisan, Ogun, Nigeria, [2]University of Ibadan, Ibadan, Oyo, Nigeria, [3]Shantou University Medical College, Shantou, Guangzhou, China.

Exposure to electrical and electronic wastes containing variety of hazardous substances is a major social problem and threat to the public health. This study investigated the potential of raw and simulated electronic waste leachates from a major e-waste dumpsite in Nigeria to induce DNA damage using the mouse bone marrow micronucleus (MN), chromosome aberration (CA) and sperm morphology assays and the alkaline comet assay on human peripheral blood lymphocytes (HPBL). The leachates and dumpsite soil were analyzed for USEPA and WHO priority organics and heavy metals. The assays were carried out at 0.5 - 50 % concentrations of the leachates. Assessment of sperm shape at 5 weeks from the first day of exposure showed significant ($p<0.05$) elevation of sperm abnormalities with significant decrease in sperm count. In the CA test, there was concentration-dependent significant ($p<0.05$) reduction of mitotic index and induction of different types of chromosomal aberrations. MN analysis showed a dose-dependent induction of micronucleated polychromatic erythrocytes across the treatment groups. In the Comet assay, the leachates induced significant concentration-dependent increase in DNA damage in HPBL as assessed by the standard Comet parameters. High concentrations of PAHs, PCBs, PBDEs and heavy metals such as Pb, Cu, Cd and Ni, were found in the tested samples. The interaction of these with the DNA in the test systems contributed significantly to the observed genetic damage. These findings indicate that e-waste contain potential somatic and germ cell genotoxins and may be of genetic risks to exposed population.

P39

Chromosomal Aberrations in Mouse Spermatogonia Following Ingestion of Fluoride and Aluminum. Rao AVB. Pondicherry University, Puducherry, India.

The present study focuses on chromosomal aberrations in male mice in order to assess the cytogenetic status of fluoride, aluminum and a combination of both. Five week old male mice with an average weight of 25 g were selected to study the mutagenicity of fluoride and aluminum. The doses were 1/5 and 1/10 the LD_{50} of fluoride mixed with 1/10 LD_{50} of aluminum in order to analyse the impact of a lower dose of aluminum on fluoride mutagenicity. Following treatments the mice were sacrificed after 30 and 60 days. Meiotic preparations were made as per standard protocols. 100 spermatocytes at the diakinesis metaphase stage of meiosis were scored for the induction of chromosomal aberrations such as autosomal, univalents, sex-univalents polyploidy, aneuploids and translocations. Animals exposed to fluoride and aluminum alone exhibited all types of aberrations including translocations. The changes in the chromosomal aberrations were dose and time dependent in fluoride treated animals. Aluminum treated animals also showed a significant incidence of chromosomal aberrations. The results indicated that 1/10 LD_{50} of aluminum is tolerable to the mouse at both exposure periods. The treatment with the combination of fluoride and aluminum exhibited cytogenetic changes that did not reach statistically significant levels, with some evidence of dose dependence. However higher dose of fluoride, combined with aluminum exhibited similar degree of chromosomal aberrations as that of fluoride alone. It is therefore suggested that aluminum may be beneficial in alleviating cytogenetic perturbations induced by low levels of fluoride.

P40

Age-Related Effects of Ionizing Radiation on Chromosome Damage in Humans. Bakhmutsky MV, Joiner MC, Tucker JD. Wayne State University, Detroit, MI, United States.

Age may be an important factor that is not always considered when performing risk assessments. The purpose of this study is to determine whether age affects an individual's susceptibility to radiation. Blood samples were collected from 10 healthy human donors, ranging from 22 to 78 years old. Samples were acutely irradiated with Cobalt 60 gamma rays to doses of 0 (control), 1, 2, 3 and 4 Gy. Blood was cultured in the presence of PHA and Colcemid was added to arrest cells in metaphase. Metaphase spreads were stained with FISH whole chromosome paints. Chromosomes 1, 2 and 4 were labeled with Cy3 and chromosomes 3, 5 and 6 were labeled simultaneously with FITC. Approximately 100 to 1000 cell equivalents per dose point (more for lower doses) per donor were scored for chromosome aberrations by trained individuals using epifluorescent microscopes. Regression analyses and ANOVA were used to determine whether dose responses showed an age-related effect based on the number of events including translocations, color junctions and dicentrics. Preliminary analyses suggest there is no age-related difference among the radiation responses in adults. Data from 10 additional adult donors are currently being collected. Umbilical cord blood samples will also be obtained and evaluated for radiation-induced chromosome damage to compare to the adult frequencies. If the absence of an age-related difference in response to ionizing radiation is retained, then risk assessments for radiation exposure could be simplified.

P41

Evaluation of p53 Genotype on Gene Expression and O^6-Ethylguanine Levels in Kidney Tissue from Male C57BL/6 Mice Exposed to ENU. Petibone DM[1], Kulkarni R[1], Chang C-W[2], Chen JJ[2], Churchwell MI[3], Beland FA[3], Morris SM[1]. [1]Division of Genetic and Molecular Toxicology, NCTR, Jefferson, AR, United States, [2]Division of Personalized Nutrition and Medicine, NCTR, Jefferson, AR, United States, [3]Division of Biochemical Toxicology, NCTR, Jefferson, AR, United States.

These studies were initiated to define further the role of p53 on the response to the DNA damaging agent N-ethyl-N-nitrosourea (ENU). Alterations in gene expression levels in kidney were determined in male, 8-9 week old, p53 wild-type (WT), heterozygous (HET), and knockout (KO) mice at 4 and 24 hours after i.p. exposure to either saline or 150 mg/kg of ENU. Total RNA was used to generate cDNA, and the gene expression levels determined utilizing a targeted qPCR p53 signaling array. In addition, the levels of O^6-ethylguanine in genomic kidney DNA were determined by LC/MS/MS. ANOVA analysis of the gene expression data obtained from both arrays revealed that exposure to ENU resulted in time- and genotype-dependent differential expression of genes involved in apoptosis and the cell-cycle. Increased expression of Bax, $p21^{cdkn1a}$, Tnfrsf10b, and Mdm2 was noted in each genotype at 4 hours after exposure, although the level of expression was not as pronounced in the HET and KO mice. Both Bax and p21 were also differentially expressed at 24 hours in the WT mice. In addition, the levels of O^6-ethylguanine were significantly higher in the KO animals than either the WT or HET mice at 24 hours after exposure. Taken together, these data suggest that in the murine kidney, DNA ethylation induces p53-modulated cell-cycle arrest through p21, and that damaged cells may be cleared through a Bax-mediated apoptotic pathway. The views expressed in this communication are those of the authors and do not necessarily reflect U.S. FDA policy.

P42

The Effects of Folic Acid Deficient and Supplemented Diets over Multiple Generations on DNA Damage in Mice. Swayne BG[1], Behan NA[2], Yauk CL[1,2], MacFarlane AJ[1,2]. [1]Carleton University, Ottawa, ON, Canada, [2]Health Canada, Ottawa, ON, Canada.

Introduction: Folate deficiency decreases *de novo* nucleotide synthesis and cellular methylation potential, which can lead to chromosome damage. Folic acid supplementation has been hypothesized to inhibit folate-dependent one carbon metabolism, which could also lead to DNA damage. Since the introduction of fortification, a significant proportion of the general population has blood folate levels indicative of high folic acid intakes. The present work examines the effects of diets supplemented or deficient in folic acid on DNA damage to address concerns surrounding fortification. Methods: The red blood cell flow-cytometry micronucleus assay was used to investigate micronucleus frequency in Balb/c mice maintained on folic acid deficient (0 mg/kg), regular (2 mg/kg) or supplemented diets (6 mg/kg) for: (a) three generations; or (b) from weaning for 15 weeks. Results: Multiple generation study: The folic acid deficient diet resulted in a statistically significant 3-fold and 2.7-fold increase ($P < 0.0001$) in micronucleus frequency in reticulocytes and erythrocytes, respectively. Weaning study: The folic acid deficient diet resulted in a significant 1.24-fold increase ($P < 0.001$) in micronucleus frequency in the erythrocytes of male mice. Discussion: Folic acid deficiency leads to an increase in chromosome damage in red blood cells of mice from weaning to maturity and over multiple generations. Folic acid supplemented diets did not cause changes in micronucleus frequency, suggesting that excess folic acid is not detrimental nor does it provide protection against chromosome damage. The data alleviate concerns relating to the clastogenic potential of chronic exposure to high dietary folic acid.

P43

Transgenerational DNA Damage Alterations in Human Cord Blood Corroborating Cigarette Smoke As the First Indentified Human Germ Cell Mutagen. Laubenthal J[1], Zlobinskaya O[2], Poterlowicz K[3], Fthenou E[4], Baumgartner A[5], Schmid TE[2], Anderson D[1]. [1]University of Bradford, Department of Biomedical Sciences, Bradford, United Kingdom, [2]Technical University of Munich, School of Medicine, Department of Radiation Therapy, Munich, Germany, [3]University of Bradford, Centre of Skin Sciences, Bradford, United Kingdom, [4]University of Crete, School of Medicine, Department of Histology, Crete, Greece, [5]University of Leipzig, Cardiac Centre, Department of Paediatric Cardiology, Leipzig, Germany.

Analyses of the relevance of preconceptional and/or prenatal toxicant exposures are difficult, since gestational exposures usually cannot be separated from preconceptional exposures in human populations. Hence, transgenerational alterations in genomic stability and DNA damage have only been investigated in radiation exposed humans so far, while transgenerational effects of lifestyle, occupational or environmental factors have solely been studied in rodents under restricted laboratory conditions. Here, DNA damage was assessed *via* the Comet assay and semi-automated confocal laser scanning microscopy of γH2AX-foci in cord, maternal and paternal blood as well as spermatozoa from 39 families of two well documented birth cohorts in Crete-Greece and the UK. Using multivariate linear regression analysis with backward selection, only preconceptional paternal and gestational maternal smoking were identified as significant predictors for DNA damage in cord blood. Active smoking was also a significant predictable for γH2AX-foci in the respective paternal blood, while in the spermatozoa and maternal blood, increased DNA strand-breaks were found. These results suggest that parental smoking metabolites can genetically or epigenetically modify (1) the male germline and (2) foetal DNA by passing through the blood-placenta barrier. These can both lead to DNA damage alterations in the unexposed offspring as shown here for human cord blood and previously in transgenerational rodent studies using the same biomarkers. Together, these data show a transgenerational role for preconceptional and gestational cigarette smoke exposure in the induction of DNA damage in the unexposed human offspring, confirming the hypothesis that cigarette smoke is the first identified human germ cell mutagen.

P44

The Effect of Advanced Paternal Age on Genetic Risks Is Mediated through Dysregulation of HRAS Signalling in the Testis. Goriely A, McGowan SJ, Pfeifer S, Itani A, McVean GAT, Wilkie AOM. University of Oxford, Oxford, United Kingdom.

Although advanced paternal age is a well known genetic risk factor, the mechanisms that mediate this effect are poorly understood. A small group of disorders, including Apert syndrome (FGFR2 mutations) and achondroplasia (FGFR3), that we collectively call 'paternal age-effect' (PAE) disorders, provides a good model to study the biological basis of PAE. Direct quantification of spontaneous FGFR2/3-associated mutations in human sperm suggests that, although occurring rarely, PAE mutations provide a selective advantage to mutant spermatogonial stem cells (SSC), resulting in their clonal expansion over time. To further characterise the mechanisms associated with PAE, we have quantified the Costello syndrome-associated mutations at HRAS codon G12 in human sperm, using massively parallel sequencing. Mutations were detected in the sperm of most men and shown to increase significantly with donor's age. The relative prevalence of G12 mutations was strikingly different to that reported in cancer: in sperm, G12S (34G>A at CpG dinucleotide) is the commonest HRAS mutation (average~1:45,000), followed by G12D (35G>A) (~1:125,000), while G12C (34G>T) and the strongly activating G12V (35G>T) are observed at lower levels (~1:300,000-1:400,000). Unexpectedly, we also identified many instances of tandem substitutions in sperm, suggesting an unusual mechanism of mutagenesis within the testis. These results suggest that PAEs are mediated through activation of the RAS pathway, which is a key determinant of SSC self-renewal. As RAS is required in many cellular contexts, dysregulation of this pathway is expected to be relevant to the pathology of other diseases, including cancers and neurocognitive disorders such as autism and schizophrenia.

P45

Keeping It Simple: Searching for Unstable Short Simple Tandem Repeats in the Germline of Mice. Beal MA[1], Glenn TrC[2], Lance SL[3], Somers CM[1]. [1]University of Regina, Regina, SK, Canada, [2]University of Georgia, Environmental Health, Athens, GA, United States, [3]Savannah River Ecology Lab, Aiken, SC, United States.

Expanded simple tandem repeats (ESTRs) are the most efficient tools for measuring germline mutation induction in mice. However, ESTR mutation detection can be imprecise and is not suitable for high-throughput assays. We are identifying unstable short simple tandem repeats (microsatellites) that may offer an improvement over ESTRs for germline mutation studies. DNA from four different populations of inbred mice (all C57/B6), as well as offspring from ENU-treated sires, was enriched for microsatellites using the following probes: $(AAAT)_8$, $(AACT)_8$, $(AAGT)_8$, $(ACAT)_8$, $(AGAT)_8$, $(AAG)_8$, $(AATG)_6$, and $(AACC)_5$. The enriched pool was sequenced using a Roche 454 system to allow comparison of a large number of captured microsatellite loci simultaneously. So far, 58 potentially variable loci have been identified: 42 tetranucleotides, 6 dinucleotides, and 10 compound microsatellites. 50% of the isolated microsatellite loci appear variable due to sequencing error, while the other 50% have different alleles both between and within populations. Candidate loci showing allelic variation are likely unstable and are being used to screen the offspring of male mice exposed to ionizing radiation (pedigree approach) to determine their utility for germline mutation studies. In a limited screening we have observed mutations at several microsatellite loci in pedigrees, suggesting that mutation rates may be as high as 10^{-2}. Current efforts are focused on screening more loci and pedigrees to: (a) confidently establish mutation frequency, and (b) determine sensitivity to mutagen exposure.

P46

Hyper-Variability in the Length of the Constitutive Heterochromatin Regions and Breast Cancer Susceptibility. Capsel KL, Wang Y, Eastmond D. Environmental Toxicology Graduate Program, University of California, Riverside, Riverside, CA, United States.

Breast cancer is the most common cancer among women. Previous studies have reported that women with increased size variability of the constitutive heterochromatin regions between homologs of chromosomes 1 and 9 are at increased risk of developing breast cancer. In studies from our laboratory, cell lines derived from breast cancer patients exhibited significantly greater variability in the size of the heterochromatin regions on chromosomes 1 and 9 than those seen in matched control cell lines ($p<0.01$). The mechanisms underlying this increase are unknown. To determine whether inefficient repair of interstrand crosslinks (ICL) may be involved, lymphoblastoid cell lines from breast cancer patients and age-, sex- and ethnicity-matched controls were exposed to the chemical agents, mitomycin C, melphalan and 2-chloroethylamine. Changes in the size of the heterochromatin were measured using FISH probes specific for the classical satellite sequences located within the constitutive heterochromatin of chromosomes 1 and 9. Results show that two out of six cell lines from the breast cancer patients show variability in the heterochromatin region of chromosome 9 that is strikingly greater following treatment with all three DNA damaging agents than in the matched controls ($p<0.01$). The size variability is not specific to agents that induce ICL as increases were seen with 2-chloroethylamine, a monofunctional alkylating agent. Interestingly, the two cell lines did not show increased sensitivity to chromosomal breakage, as chemically induced micronucleus frequencies were not elevated as compared to the matched controls. Future studies involve identifying the significance and mechanisms underlying the observed hyper-variability.

P47

Lack of Energy-Induced by Sodium Azide Interferes with Differentiation of Organs and Tissues. Ramos P, Hernandez BR. UNAM, Mexico, DF, Mexico.

Introduction. Human exposure to the metabolic poison, Sodium Azide (SA) has increased gradually as new applications are discovered: preserving biological fluids, blocking specific enzymes, activating the automobile airbags and many more. Although SA mutagenic activity in bacteria was known since 1948, the information about its effects in eukaryotic systems is rather scarce and contradictory, probably due their different metabolic pathways involved. In Drosophila melanogaster, SA induces mitotic recombination and interferes with the reproductive activity of adults exposed chronically during larval development. The aim of this study is to compare the toxicity of SA in two types of flies differing in their capacity to activation and detoxification of xenobiotics. Methods. Third instar, Wild Type (WT) and Insecticide Resistant (ORR) larvae were used. From 20 mM SA, successive dilutions were prepared using 5% sucrose as dissolvent. Groups of 50-100 larvae were put inside tubes containing cellulose (60 mg) and 1 ml of SA solution. After 6h, larvae were washed with tap water and transferred to vials with fresh medium. Adults recovered were sexed, counted and preserved in 70 % ethanol for morphological examination. Results. Medium and high concentrations assayed affected similarly the WT and ORR flies but the lower concentrations were toxic for ORR flies. Malformed organisms were recovered mainly from ORR flies. Discussion. The differential metabolism of the ORR flies could enhance the activation of metabolites and hence interfere with the differentiation process through development. Acknowledgment: For technical support to H.Rivas and students (Introduction to the Genetic and Environmental Toxicology Workshop).

P48

Transgenerational Effects of Chemicals by Mutagenicity and/or Epimutagenicity. Shibuya T, Horiya Y. "Tox21" Laboratory, Yugawara, Kanagawa, Japan.

Various chemicals can mutagenize germ cells of experimental animals at various stages of both sexes. Nevertheless, only several chemicals have been reported to have a potency to mutagenize stem cell spermatogonia (SCG) until now. As SCG are very important germ cell stage to assess genetic effects on male animals, but the mutation rates induced by these chemicals are very low. On the other hand, we already reported that ENU induces mutations in primordial germ cells (PGC) of male mice with higher mutation rate than in SCG (Shibuya *et al*., 1993, 1996). Therefore, PGC are more important germ cell stage than SCG to evaluated genetic effects by chemicals. Transgenerational effects (TG) through epimutation by various chemicals, such as endocrine disrupting chemicals, alkyating agents and various nutritional conditions have been reported. Skinner *et al.* (2005) reported that Vinclozolin induces various TG effects by epimutation for 4 generations when treated with rat PGC. The induced epimutation rate by these chemicals are far higher than those by mutagenicity. Nagao *et al.* (1994, 1999) already reported that the incidence of teratogenicity by mutagenic chemicals show about two order of magnitude high than those in SCG. Shibuya *et al.* (2003) reported that the rate of intragenic recombinations originated from a kind of chromatin configuration are far high than those of point mutations. Nowadays, on the TG effects by chemicals through epimutational events may have greated impact than those by mutations.

P49

Timing of Early Developmental Cigarette Smoke Exposure Determines Epigenetic and Gene Expression Changes. Lyon JL, Gordon T. New York University School of Medicine, Tuxedo, NY, United States.

Introduction: The CDC estimates 2 million babies per year are exposed to cigarette smoke (CS), a complex, carcinogenic mixture, during early development. CS is known to alter histone modifications and the methylation patterns of DNA. CS exposure during the delicate periods of fetal and neonatal development may change the expression of critical genes. Methods: Pregnant C57BL/6J mice were exposed to 5 mg/m^3 mainstream CS for 3 hrs/day, 5 days/week until birth. For postnatal exposures, 4 day old mice were exposed to 5 mg/m^3 ETS for 3 hrs/day, 5 days/week, until day 21 post-partum. Mice were sacrificed at 23 days and 11 weeks of age. Microarrays were used to analyze differential gene expression and results confirmed using RT-RT PCR. Global methylation was analyzed with a microplate based kit. Histone modifications potentially important for the altered gene expression were examined using chromatin immunoprecipitation and qPCR. Results: Upregulation of a DNA repair gene growth arrest and DNA damage inducible gamma 45, a glucose regulating gene glucose-6-phosphatase, catalytic, and an important gene in the circadian rhythm pathway, aryl hydrocarbon receptor like, was found in both exposures at 23 days and 11 weeks of age. Global methylation analysis revealed hypomethylation in the prenatal exposure but hypermethylation in the postnatal exposure at 23 days, but no difference was seen at 11 weeks. Discussion: Early developmental exposures to CS alter gene expression patterns, however, epigenetic effects of the exposure vary depending on the developmental period in which the exposure occurred.

P50

Epigenetics of Parental Exposure to Environmental Mutagens. Dubrova YE. Department of Genetics, University of Leicester, Leicester, United Kingdom.

Recent studies have established that epigenetic changes play an important role in many common human diseases, including cancer. Given that the epigenetic landscape of the mammalian cell is not fixed and undergoes massive reprogramming during development, it can potentially be affected by a variety of environmental factors. As the majority of the *de novo* epigenetic marks, including DNA methylation, are faithfully reproduced during DNA replication, they are transmissible through many cell divisions and, in some cases, can be passed from parents to their offspring. An increasing body of experimental evidence from animal and human studies suggests that environmentally-induced epigenetic changes can be inherited by subsequent generations and can result in transgenerational phenotypic alterations, including predisposition to common diseases. Our recent data on radiation-induced transgenerational instability in the mouse germline show that the effects of exposure to mutagens may not be restricted to the directly affected parental germ cells, but can also manifest in their non-exposed offspring. Our results show that mutation rates are substantially elevated in the germline and somatic tissues of first- and second-generation offspring of male mice exposed to ionizing radiation, chemical mutagens and anticancer drugs. This remarkable transgenerational destabilization is attributed to the presence of a subset of endogenous DNA lesions, caused by epigenetic changes affecting the pattern of gene expression in the offspring. The potential implication of these results for the estimates of genetic risk will be discussed.

P51

Synergistic Effect of Coffee and Radicals Generated by Microwave Detected by Tradescantia- Microspore-Micronucleus Bioassay. Ma TH. Western IL University, Macomb, IL, United States.

Tradescantia micronucleus in microspore mitotic division was used to study the synergistic effect of coffee solution and radicals generated by microwave heating process. Four groups of Tradescantia inflorescences were maintained in 1) Tapwater, 2) Coffee soltion at 1/4 dilution of regular strength, 3) Coffee solution pre-exposed to mirowave, 4) Water pre-exposed to mircowave. Both coffee soluiton and water used for treatment were at 25 degree C. Results of micronuclei frequencies of these 4 groups were: 1) Tapwater = 0.174 MN/Cell; 2) Coffee solution = 0.183MN/Cell; 3) Coffee + radicals = 5.00/Cell; and 4) Water + radicals = 1.22 MN/Cell. These preliminary data show the potenitial deleterious effect of synergism of microwave heated coffee for the dialy pleasure with a cup of good morning hot coffee.

P52

Cytotoxicity and the Validation of the 96-Well *In Vitro* Micronucleus Assay Using Flow Cytometry with Chinese Hamster V79 Lung Cells. Murray JA, Sonders PA, Nicolette JJ. Abbott Laboratories, Abbott Park, IL, United States.

The *in vitro* micronucleus assay is a relatively easy, inexpensive and quick way to determine if a compound has potential to cause chromosome damage. We recently investigated increasing the throughput of the assay for pharmaceutical discovery support from a 24-well design to a 96-well format. During our validation, we tested several commercially available compounds that have published micronucleus results detailing the mechanisms of chromosome damage. Although overall results are concordant with our 24-well assay, cytotoxicity is an increasing disadvantage. Appropriately identifying cellular toxicity is critical to avoid repeat analysis in a time-critical environment or inappropriately evaluating excessively toxic conditions that can lead to false positive results. To support validation of the 96-well format, we will discuss the utilization of commercially available non-lethal cytotoxicity assays, and compare them with current methods.

P53

Flow Cytometric Scoring of TK6 Micronuclei in 96-Well Plates: Interlaboratory Ring Trial Contribution. Bryce SM, Avlasevich SL, Dertinger SD. Litron Labs, Rochester NY, United States.

Flow cytometric methods (*In Vitro* MicroFlow®) for scoring micronuclei (MN) *in vitro* [*EMM* (2006) 47:56-66] were modified and miniaturized so that all procedures were accomplished in the same 96 well plate. The results described herein were generated at Litron, and are part of a ring trial that involves 4 laboratories. TK6 cells were treated continuously for ~24 hrs with 8 clastogens, 8 aneugens, 8 non-genotoxicants, and 8 non-genotoxicants considered to generate irrelevant positive findings. Inclusion of a consistent number of fluorescent latex beads provided several cytotoxicity endpoints from pre- and post-treatment Cell to Bead ratios: percent relative survival (%RS-C:B), percent relative population doublings (%RPD), and percent relative increased cell count (%RICC). Concurrent with MN enumeration, Nuclei to Bead ratios were used to derive percent relative survival values (%RS-N:B). Assessment of assay performance was based on a positive call criterion: ≥ 3-fold increase in mean MN frequency relative to concurrent vehicle control, with a 55% cytotoxicity limit. The percentages of genotoxicants correctly identified were 87.5%, 68.75%, 50%, and 87.5% for RS-C:B, RPD, RICC, and RS-N:B, respectively. The percentage of non-genotoxicants (including artifactual positives) correctly identified were 87.5%, 87.5%, 93.8%, and 87.5% for RS-C:B, RPD, RICC, and RS-N:B, respectively. Additional experiments examining the effects of several apoptosis-inducing chemicals will also be discussed. These data suggest that this miniaturized flow cytometry-based *in vitro* MN assay is capable of good performance, but that cytototoxicity endpoint(s) require careful consideration, since they can markedly affect sensitivity and specificity.

P54

Dose-Response Evaluation of Micronucleus and p53 DNA Damage Response by Methyl Methanesulfonate, Etoposide, and Quercetin. Sun B[1], Ross S[1], Thomas A[1], Trask J[1], Andersen M[1], Carmichael P[2], White A[2], Clewell R[1]. [1]The Hamner Institutes for Health Sciences, Durham, NC, United States, [2]Unilever SEAC, Bedfordshire, United Kingdom.

Proliferating cells respond to chemical induced DNA damage by initiating the p53 pathway, leading to cell cycle arrest, damage repair or apoptosis. The current study used the HT1080 human fibrosarcoma cell line to examine dose-response for DNA damage and cellular response after exposure to chemicals with different mechanisms of genotoxicity: methyl methanesulfonate (MMS), etoposide (ETP) and quercetin (QUE). Cell cycle analysis demonstrated that significant G2/M phase arrest is induced by the DNA topoisomerase II inhibitor ETP at doses > 0.02 µM, while QUE, a chemical which causes oxidative damage at high doses, induced a moderate level of S-phase arrest at lower doses (> 10 µM) and a strong G1 arrest at higher concentrations (>30 µM). MMS, an alkylating agent, induced a strong S phase arrest, but only at high doses (> 100 µM). All three chemicals dose dependently increased p-H2AX, p53 and p-p53, though much lower concentrations of ETP were required than QUE or MMS. ETP significantly increased micronucleus formation at doses similar to those required for activation of p53, however QUE and MMS only moderately induced micronucleus formation. ETP, QUE and MMS have distinct effects on p53 pathway activation, cell cycle arrest and micronucleus formation, and continued investigation into the dose-response for protein and gene changes will provide insight into the determinants of cellular response to chemicals with different mechanisms of DNA damage.

P55

Involvement of p53 Function in Different Magnitude of Genotoxic and Cytotoxic Responses in *In Vitro* Micronucleus Assays. Hashimoto K, Nakajima Y, Uematsu R, Matsumura S, Chatani F. Takeda Pharmaceutical Company Limited, Fujisawa, Kanagawa, Japan.

In *in vitro* micronucleus (MN) assays the sensitivity to MN induction or cytotoxicity can vary depending on the kind of cells employed. This study was conducted to examine the involvement of the p53 function in the different sensitivities between Chinese hamster lung (CHL) cells and human lymphoblastoid TK6 cells in MN assays. MN induction and cytotoxicity were compared using MN-inducing chemicals reported as DNA reactive clastogens, non-DNA reactive clastogens or aneugens. The study revealed that the maximum levels of MN induction in p53-compromised CHL cells were higher than those in p53-competent TK6 cells, but MN were significantly induced in TK6 cells at lower concentrations than in CHL cells. Most of the test chemicals produced a more severe cytotoxicity in TK6 cells, suggesting TK6 cells are more sensitive for cytotoxicity than CHL cells. An additional experiment with 9 MN inducers revealed that the magnitude of MN induction and cytotoxicity were comparable between p53-competent TK6 cells and its p53-null mutant NH32 cells at the same concentrations. Furthermore, the MN frequencies induced by methylmethane sulfonate, aphidicolin and hydroxyurea in NH32 cells were identical to those in TK6 cells at different recovery times. From these results, it is suggested that the p53 abrogation does not explain the difference in sensitivity to MN induction or cytotoxicity between CHL and TK6 cells. In this regard, p53 abrogated NH32 cells can be an option for the *in vitro* MN assay.

P56

Replication Stress Induces a Novel Class of Micronuclei That Are Marked by Phosphorylation of H2AX. Xu B, Sun Z, Gong Y, Shao C. Shandong University, Jinan, Shandong, China.

Micronuclei (MN) in mammalian cells serve as a reliable biomarker of genomic instability and genotoxic exposure. Elevation of MN is commonly observed in cells bearing intrinsic genomic instability and in cells exposed to genotoxic agents. Phosphorylation of H2AX, or γ-H2AX, marks the presence of double-strand breaks (DSBs) in DNA. By immunofluorescence staining of MN using anti-γ-H2AX antibody, MN can be divided into MN-γ–H2AX (+) and MN-γ–H2AX (-). Using chemical treatment and RNA interference, we demonstrated that the MN-γ–H2AX (+) can be preferentially induced by replication stress. First, the frequency of MN-γ-H2AX (+) can be efficiently elevated by the DNA replication stressors (hydroxyurea, aphidicolin and thymidine), but not by paclitaxel that does not cause DNA replication stress. Second, by examining the distribution of the two types of MN in cells at different stages of cell cycle, we showed that MN-γ–H2AX (+) were formed during S phase. Third, in cells that experience a prolonged S phase due to the depletion of CUL4B by RNAi, only MN-γ–H2AX (+), but not MN-γ–H2AX (-), was significantly induced. Finally, in cells that were depleted of RPA1 by RNAi, and therefore were encountering DNA replication stress, the frequency of MN-γ–H2AX (+) is significantly increased. Thus, MN-γ-H2AX (+) are probably derived from chromatin containing DSBs caused by replication stress. Our findings suggest that MN-γ–H2AX (+) may serve as a more sensitive marker for exposure to genotoxic agents that cause replication stress and for genomic instability associated with DNA replication errors.

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***In Vitro* Micronucleus Assay with 5-Fluorouracil: Is Extended Recovery Time Necessary?** Farabaugh CS, Wells MM, Moy ML, Schlosser MJ. WIL Research Company, Skokie, IL, United States.

Published reports indicate that 5-fluorouracil (5-FU), a nucleoside analogue, produces variable responses in the *in vitro* micronucleus assay under a variety of treatment conditions with and without Cytochalasin B (CytB) in human lymphocytes, TK6 cells, CHO cells, CHL cells, and L517Y cells, with 0- to 45-hour recovery periods (Lorge *et al.* 2006; Kirkland *et al.* 2010). This laboratory has conducted the *in vitro* micronucleus assay with TK6 cells using both conventional and flow cytometric methods, along with several different cytotoxicity measures, to determine if an extended recovery is necessary for micronucleus formation following incubation with 5-FU. TK6 cells were suspended in complete media (RPMI-1640 medium supplemented with a final concentration of 10% (v/v) heat-inactivated horse serum, 2 mM L-glutamine, and 100 units/mL penicillin and 100 µg/mL streptomycin) at 0.5 - 2.0 × 10^5 cells/mL and seeded into 25 cm^2 flasks (5 mL/flask). All cultures were incubated at 37 ± 1°C in vented flasks in a humidified atmosphere of 5 ± 1% CO_2. When required, CytB was used at 6 µg/mL. Cultures were either harvested 24-hours post-treatment (24-0), or the test article was removed at 24-hours post treatment and harvested after a 24-hour recovery period (24-24). Slide analysis indicated statistically significant increases in micronuclei at approximately 55% cytotoxicity with CytB (24-0) and negative responses without CytB (24-24). Flow cytometry analysis without CytB (24-24) indicated negative or borderline results using cytotoxicity measurements including relative population doubling (RPD), relative increase in cell count (RICC), and Litron Laboratory's relative survival (RS) calculation.

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Analysis of Cell Proliferation, Apoptosis and MN Induction with *In Vitro* Flow Cytometry Primary Human Lymphocyte Micronucleus Assay. Elhajouji A[1], Lukamowicz M[1], Kirsch-Volders M[2], Suter W[1]. [1]Novartis Institutes for Biomedical Research, Basel, Switzerland, [2]Vrije Universiteit Brussel, Brussels, Belgium.

In order to minimize the number of positive *in vitro* cytogenetic results which are not confirmed in rodent carcinogenicity tests biological systems which are p53 and DNA repair proficient are recommended. Moreover an appropriate cytotoxicity parameter for the top dose selection should be considered. The recent International Conference on Harmonisation draft S2 and Organisation for Economic Co-operation and Development (OECD) 487 guidelines accepted the *in vitro* micronucleus test (MNT) as a valid alternative method for *in vitro* chromosome aberration test within the *in vitro* cytogenetic test battery. Since mitosis is a prerequisite for expression of the micronuclei, it is compulsory to demonstrate that cell division occurred, and if possible, to identify the cells that completed mitosis. The OECD guideline recommends the use of a cytokinesis block for the assessment of proliferation in primary human T-lymphocytes. Our project was initiated to develop a novel flow cytometry based primary human lymphocyte MNT method. This new assay is based on a three step staining procedure: Carboxyfluorescein succinimidyl ester as a proliferation marker, ethidium monazide for chromatin of necrotic and late apoptotic cells discrimination and 4, 6-diaminodino-2-phenylindole as a DNA marker. The "proof of principle" of the method was performed using genotoxic and non-genotoxic compounds: Methyl methanesulfonate, Mitomycin C, Vinblastine sulfate, Cyclophosphamide, Sodium Chloride and Dexamethasone. It has been shown that the new flow cytometry based primary human lymphocyte MNT method is at least an equally reliable method as the standard Cytochalasin B MNT.

P59

Development of a GLP 3-D EpiDerm™ Reconstructed Human Skin Micronucleus Assay. Shi J, Szkudlinska A, Wang K, Roy S, Hickman S, Madraymootoo W, Aardema M. BioReliance Corporation, Rockville, MD, United States.

The 3-D reconstructed human skin micronucleus assay (RSMN) in EpiDerm™ is a promising new assay for the assessment of genotoxicity. Analysis of micronuclei in primary human skin offers a more biologically relevant *in vitro* approach to assess genotoxicity for many types of dermal exposures including drugs, chemicals and cosmetics, based on the fact that skin is the site of maximum exposure for dermal applications. EpiDerm™ provides a functional stratum corneum that takes into account permeability, and is expected to have normal dermal metabolic capability, normal DNA repair and cell-cycle control. These features are important for reducing the induction of "misleading" positive results, *e.g.* positive results that do not occur in an *in vivo* assay or carcinogenicity assay. The RSMN assay also provides an approach for following up chemicals that are positive in current *in vitro* genotoxicity assays and is especially useful for cosmetics that can no longer be tested in *in vivo* assays according to the 7th Amendment to the EU Cosmetics Directive. To meet the increasing interest in this assay, we have developed a GLP RSMN assay. Results for model chemicals including mitomycin C and vinblastine sulphate show dose-dependent increases of MN and demonstrated good reproducibility and comparability to previously published results. Studies are ongoing with genotoxins and nongenotoxins with various modes of action.

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Evaluation of Chemicals Requiring Metabolic Activation in the EpiDerm™ 3-D Human Reconstructed Skin Micronucleus (RSMN) Assay. Aardema MJ[1], Barnett BC[2], Mun G[3], Dahl EL[4], Curren RC[3], Hewitt NJ[5], Pfuhler S[2]. [1]Marilyn Aardema Consulting LLC, Cincinnati, OH, United States, [2]The Procter & Gamble Co, Cincinnati, OH, United States, [3]Institute for InVitro Sciences, Rockville, MD, United States, [4]Exponent, Alexandria, VA, United States, [5]Nicky Hewitt Scientific Writing Services, Erzhausen, Germany.

The *in vitro* human reconstructed skin micronucleus (RSMN) assay in EpiDerm™ is a promising new assay for evaluating genotoxicity of dermally applied chemicals. A global prevalidation project sponsored by the European cosmetics companies trade association (COLIPA), and ECVAM, the European Center for Validation of Alternative Methods, is underway. Studies to date have focused on chemicals that do not require metabolism and the results demonstrate good inter-laboratory and inter-experimental reproducibility. We have expanded these studies to investigate chemicals that require metabolic activation: 4-nitroquinoline-n-oxide (4NQO), cyclophosphamide (CP), dimethylbenzanthracene (DMBA), dimethylnitrsoamine (DMA), dibenzanthracene (DBA) and benzo(a)pyrene (BaP). The protocol was extended to three applications over a 72 h treatment period and compared to the standard 2 treatments over 48 h. For 3/6 chemicals, the results were the same between the 48 h and 72 h treatments [DMBA positive both 48 and 72 h; DBA and DMN negative both 48 and 72 h]. 4NQO was negative with the standard 48 h treatment but positive with 72 h. CP had mixed results at the 48 h, but was positive with 72h. BaP was weakly positive with the 48h treatment and negative with the 72 h treatment. While further work with chemicals that require metabolism is needed, at this point in time, for general testing, it is prudent to use a longer treatment period in situations where the standard 48 h treatment is negative or questionable.

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Flow Cytometric Approach to Scoring of Micronuclei and Cell Cycle Status in the Reconstructed Human Skin Micronucleus Assay. Sullivan AA[1], Crosby ME[1], Mun GC[2], Bryce SM[3], Pfuhler S[1]. [1]The Procter & Gamble Company, Cincinnati, OH, United States, [2]Institute for In Vitro Sciences, Gaithersburg, MD, United States, [3]Litron Laboratories, Rochester, NY, United States.

The *in vitro* reconstructed skin micronucleus (RSMN) assay is a follow-up tool for compounds with dermal exposure, which were positive in the *in vitro* standard battery. However, the manual evaluation of keratinocyte preparations on slides is very labor intense with high cost and long assay time and has limited statistical power. We have investigated the feasibility of scoring keratinocyte preparations using flow cytometry based on the Litron *In Vitro* MicroFlow® system (96h protocol). Using two model compounds, the aneugen vinblastine (VB) and the DNA crosslinker mitomycin C (MMC), we established a new protocol enabling sensitive detection of micronuclei and cell cycle status. In the standard RSMN assay [48 h exposure in the presence of cytochalasinB (cytoB) and manual evaluation] ~3 ug/tissue MMC is required to induce a significant increase in MN, while only about one third of this dose was required to generate similar effects when this 96h protocol (no cytoB, measured by flow) was used. VB, tested with the 96h protocol and measured by flow, showed a >5-fold increase at 0.0015 ug/tissue. Notably, genotoxicity at this dose as measured by the standard protocol is undetectable. Although cytoB allows for identifying divided cells, cell cycle regulation can also be monitored *via* propidium iodide staining. The results of this study indicate that the described methods simplify the evaluation of micronuclei in the RSMN, increase its sensitivity and statistical power as 20x more nuclei are scored, and allow for the generation of valuable cell cycle information. Studies demonstrating interlaboratory reproducibility are ongoing.

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Evaluation of the Repeated Dose Liver Micronucleus Assay in Rats: Summary of the Collaborative Study by CSGMT/JEMS.MMS. Hamada S[1], Takashima R[1], Shimada K[2], Zaizen K[3], Kawakami S[4], Tanaka J[5], Matsumoto H[6], Nakai T[7], Suzuki H[8], Matsumura S[9], Sanada H[10], Inoue K[11], Muto S[12], Hagio S[13], Hayashi A[14], Takayanagi T[15], Ogiwara Y[16], Maeda A[17], Narumi K[18], Takasawa H[1], Ogawa I[13], Ohyama W[18], Nakajima M[5], Morita T[19], Kojima H[19], Hayashi M[5], Honma M[19]. [1]Mitsubishi Chemical Medience Corporation, Kamisu, Ibaraki, Japan, [2]Astellas Pharma, Osaka, Japan, [3]Astellas Research Technologies, Osaka, Japan, [4]Asahi Kasei Pharma Corporation, Shizuoka, Japan, [5]Biosafety Research Center, Shizuoka, Japan, [6]Food and Drug Safety Center, Kanagawa, Japan, [7]Hokko Chemical Industry, Kanagawa, Japan, [8]Ina Research, Nagano, Japan, [9]Kao Corporation, Tochigi, Japan, [10]Kaken Pharmaceutical, Shizuoika, Japan, [11]Maruho, Kyoto, Japan, [12]Mitsubishi Tanabe Pharma, Chiba, Japan, [13]Nissan Chemical Industries, Saitama, Japan, [14]Shin Nippon Biomedical Laboratories, Kagoshima, Japan, [15]Suntory Business Expert, Osaka, Japan, [16]Taisho Pharmaceutical, Saitama, Japan, [17]Toray Industries, Kanagawa, Japan, [18]Yakult Honsha, Tokyo, Japan, [19]National Institute of Health Sciences, Tokyo, Japan.

We reported that micronucleus induction by a hepatocarcinogen can be detected in rat liver treated for 14 days or more, even at a low dose. Simultaneously, two structural isomers, 2,4-diaminotoluene (hepatocarcinogen) and 2,6-diaminotoluene (noncarcinogen) were examined in the repeated dose liver MN assays. An increased incidence of MN was observed by 2,4-DAT but not 2,6-DAT, suggesting that the repeated dose liver MN assay can specifically detect hepatocarcinogens and this assay might become one of the most useful genotoxicity test integrated into general toxicity studies. Recently, a joint research in the CSGMT was conducted to confirm the inter-facility variability and stable data acquisition in this assay. The research was supported by 18 Japanese facilities, where a 14-day repeated dose MN assay of diethylnitrosoamine was conducted. Consequently, significant and dose-dependent increases in MN frequency were confirmed in all 18 facilities. It was confirmed that the repeated dose liver MN assay is sufficiently sensitive to the liver carcinogen even at a low dose, and stable data can be obtained with little inter-facility variability.

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Structural and Numerical Aberration Inducers in Rat Liver Micronucleus Test. Itoh S, Hattori M, Nagata C, Sanbuissho A. Daiichi Sankyo Co., Ltd., Shizuoka, Japan.

The liver micronucleus test is an important method to detect pro-mutagen such as active metabolite not reaching bone marrow due to its short lifespan. We have already reported that partial hepatectomy (PH) before the administration of the test compound, is essential to detect genotoxicity of numerical chromosome aberration inducer in mice [*Mutation Res*, 632 (2007) 89-98]. In naive rats, the percentage of binucleated cells in cell classification is less than 50% of that in mice which suggests species difference in sensitivity to chromosome aberration inducers. In the present study, we investigated the response to structural and numerical chromosome aberration inducers in rat liver micronucleus test. Two of each inducers, diethylnitrosamine (DEN), 1,2-dimethylhydrazine (1,2-DMH), colchicine (COL) and carbendazim (CBZ) were used. PH was performed a day before or after the administration of the test compound in 8 weeks old male F344 rats and hepatocyte was isolated for 4 days after the PH. As a result, DEN and 1,2-DMH, a structural chromosome aberration inducer, showed significant increase in micronucleated hepatocyte (MNH) frequency in both PH before and after the administration. COL and CBZ, a numerical chromosome aberration inducer, showed significant increase in MNH frequency only in PH before administration, the same as in mice. COL increased in MNH frequency on 4 days after the PH in rats, although that induction in mice was observed on 8-10 days after the PH. PH before the administration of the test compound is essential to detect genotoxicity of numerical chromosome aberration inducer in rats.

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Adapting the Buccal Micronucleus Cytome Assay for Use in Wild Birds: Age and Sex Affect Background Rates in Pigeons. Shepherd GL, Somers CM. University of Regina, Dept. of Biology, Regina, SK, Canada.

Micronucleus (MN) formation has been used extensively as a biomarker of genotoxic damage from environmental exposures. The buccal MN cytome (BMCyt) assay provides a sensitive and non-invasive means of quantifying MN frequency in humans, but it has not been developed for use in wildlife. We adapted the BMCyt assay for use in wild birds, with a focus on feral pigeons (*Columba livia*) as a potential indicator species. Five out of six common urban bird species sampled using standard collection techniques for humans produced sufficient buccal cells for the BMCyt assay. Pigeon cells were successfully stained and scored following published BMCyt assay protocols for humans, but with a modified fixation approach using heat and methanol rather than ethanol and glacial acetic acid. Overall, pigeons had a background rate of MN formation of 0.88 MN/1000 cells. Adult pigeons had on average a 3-fold higher rate of MN formation than juveniles, and males had a 1.4- to 2.2-fold higher rate than females. Domestic and feral pigeons did not differ in overall MN frequency. Our data show that the buccal MN cytome assay can be used on wild birds, and could provide a means of assessing environmental genotoxicity in sentinel species. However, bird sex and age are important factors affecting background MN frequency, and thereby the design of environmental studies.

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Genotoxicity of Doxorubicin in F344 Rats by Combining the Comet Assay, Flow Cytometric Peripheral Blood Micronucleus Test and Pathway-focused Gene Expression Profiling. Manjanatha MG, Bishop ME, Lyn-Cook LE, Ding W. U.S. Food and Drug Administration, National Center for Toxicological Research, Jefferson, AR, United States.

Doxorubicin (DOX) is a chemotherapeutic drug effective against human malignancies such as leukemia, lymphoma and other solid tumors. Although DOX is an effective drug, its clinical efficacy is greatly limited because of severe cytotoxic and genotoxic effects in cardiac and other tissues. To evaluate the genotoxicity of this drug, approximately 7 weeks-old, male F344 rats were administered intravenously 1, 2 and 3 mg/kg bw DOX at 0, 24, 48 and 69 hr and the alkaline and enzymes-modified Comet assays in heart, kidney, and testes and micronucleus (MN) assay in the peripheral blood (PB) erythrocytes using flow cytometry were conducted. As positive controls for the MN and Comet assays, rats received three 10mg/kg/day doses of Cyclophosphamide by gavage at 0, 24 and 48 hr and a single dose of 100 mg/kg methyl methanesulfonate by gavage at 69 hr, respectively. Rats were euthanized at 72hr and 150 ul PB was removed for the MN assay and single cells were isolated from liver, heart, kidney, and testis for the Comet assays. None of the doses of DOX induced a significant DNA damage in any of the tissues examined by the alkaline Comet assay. Contrastingly, the hOGG-1-modified Comet assay showed a significant and dose dependent increase in DNA damage in the cardiac tissue whereas only the high dose was positive in the liver ($p \leq 0.05$). Studies on MN assay and gene expression profiling are ongoing. The preliminary result suggests that DOX may induce genotoxicity through free radical production.

P66

Genotoxic Evaluation of Acylated Cyclic Peptide Fragment III from Cobra Cardiotoxins on Human Lymphocytes through CBMN Cytome and Comet Assays. Gothke S, Dudley R, Vaglenov A. University of Findlay, Findlay, OH, United States.

The aim of this study was to investigate the genotoxic effects of peptide fragment derived from constituents of cobra venom on human lymphocytes after *in vitro* exposure. Cardiotoxins have not been investigated *in vitro* for genotoxicity. Evidence suggests that cardiotoxins are capable fo generating reactive oxygen species and oxidative stress leading to cell death. Moreover it has been shown that cardiotoxins exhibits toxicity against cancer cells. The cytokinesis-block micronucleus cytome, as well as the comet assays were used as endpoints. Biomarkers evaluated include: proliferative index, micronuclei, nuclear bridges, nuclear buds, and the number of apoptotic and necrotic cells. Comet slides were analyzed using the Comet IV capture system (Perceptive Instruments). The head (HI), tail intensity (TI), and olive tail moment (TM) were measured for each nucleus scored. Peripheral human lymphocytes were exposed to different concentrations of cardiotoxin fragment as follows: 0.5, 1, 2, 4, 8, and 10 mkg/ml. A comparison of biomarkers has been done with bleomycin and H_2O_2. After exposure to cardiotoxin peptide fragment, all endpoint levels of CBMN cytome assay showed similar high positive results. The MN yield triples that of the control level after exposure to the lowest dose of 0.5 mkg/ml. The higher doses induced similar levels of MN without dose-dependence from cardiotoxin concentration. The TI and TM mean values were sgnificantly higher than those of negative control results and increased similarly to CBMN cytome assay mode of action. Our results clearly demonstrated that the peptide has a strong genotoxic effect on human lymphocytes.

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The Frequency of MN and the t(15;17) in Peripheral Lymphocytes of Workers Poisoned by Benzene in São Paulo, Brazil. Santos DNC[1], Costa DF[1], Souza IC[2], Leite KR[2], Gattás GJF[1]. [1]Medical School, University of São Paulo, São Paulo, SP, Brazil, [2]Sirio Libanes Hospital, São Paulo, SP, Brazil.

Chronic exposure to high concentrations of benzene is associated with high incidence of myelodysplastic syndrome and acute myelogenous leukemia. The aim of this study was to evaluate the micronuclei frequency (CBMN) and t(15;17) by fluorescent *in situ* hybridization (FISH) in peripheral blood lymphocytes of 20 workers with clinical diagnostic of benzene poisoning after different periods of occupation in oil refinery (12 individuals) and steel industry (8 individuals). The 20 exposed (46.8 + 9.32 yo) were compared to 20 unexposed blood bank volunteers (45.7+8 yo). The micronuclei frequency observed in 20.000 binucleated cells of exposed workers (1.75 ± 2.15) was higher than that observed in the 20.000 cells analyzed in the controls (0.65 ± 0.74) and the difference was statistically significant (p=0.036). It was also observed that the MN frequency was higher in workers exposed to benzene for more than 10 years (n=13, p=0.02). The presence of at least one fusion representing the translocation identified in acute promyelocytic leukemia t(15;17) was higher in the peripheral lymphocytes of the exposed group (9.78 ± 9.54) when compared to the controls (3.94 ± 3.17), and the difference was considered significant (p=0.0194). Moreover the exposed group presented a smaller number of cells with normal FISH signal, compared to the controls (260.0 ± 10.8; 270.4 ± 7.0; p =0.0014, respectively). In summary, the MN frequency and the t(15;17) assessed by FISH could be important biomarkers to evaluated the possible genotoxic effects caused by benzene in occupationally exposed workers.

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Neutron Radiation-Induced Bystander Effect in a Normal Human Lymphoblastoid Cell Line Measured Using the Cytokinesis Blocked Micronucleus (CBMN) Assay. Seth I, Joiner MC, Tucker JD. Wayne State University, Detroit, MI, United States.

The bystander effect (BE) has been observed repeatedly in mammalian cell lines in response to photon irradiation. However, because neutrons do not induce oxidative damage to the extent seen by photons, we sought to determine whether neutrons also induce a BE. Here we used normal human lymphoblastoid cell lines that we had previously shown to exhibit the BE when cultured with irradiated-cell conditioned media (ICCM) from cells exposed to photons. Here, cells were exposed to d(48.5)-Be neutrons from a cyclotron, and culture media from irradiated cells was transferred onto cells that had not been irradiated. The CBMN assay was used to quantify the extent of genetic damage in radiation-naïve cells exposed to ICCM from cultures that had been exposed to 0.5, 1, 1.5, 2, 3, or 4 Gy neutrons. Compared to cells grown in conditioned media from unirradiated cells, all 6 ICCM cultures showed increases in micronuclei (chi-squared = 17.9, $p < 0.007$). As a pooled group, cells grown in ICCM showed 36% more MN than cells grown in conditioned media from unirradiated cells. These results suggest that neutrons induce a weak BE. However, the neutron beam has a photon dose contamination of 6%, which could imply that these photons are solely responsible for the BE, or that the BE might be an additive effect of photons and neutrons. Future experiments will distinguish among these possibilities by irradiating cells with photons at doses equivalent to the percent contamination for each of the neutron doses used here.

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Cytogenetic Analysis by FISH and Micronuclei in Mothers and Their Newborns in Biomonitoring Studies in the Czech Republic. Rossnerova A[1], Spatova M[1], Pastorkova A[1], Tabashidze N[1], Balascak I[2], Veleminsky, Jr. M[3], Rossner, Jr. P[1], Solansky I[1], Sram RJ[1]. [1]Institute of Experimental Medicine AS CR, Prague, Czech Republic, [2]2nd Medical Faculty, Charles University, Prague, Czech Republic, [3]Hospital Ceske Budejovice, a.s., Ceske Budejovice, Czech Republic.

The impact of air pollution on the level of chromosomal aberrations was studied in a group of 220 mothers and their newborns. Blood samples were collected in the winter seasons of 2007/2008 and 2008/2009 for fluorescence *in situ* hybridization (FISH) and for automated image analysis of micronuclei (MN), respectively. FISH analysis with whole-chromosome painting for chromosome #1 and #4 to assess the levels of stable and unstable chromosomal aberrations was used in 42 mothers and their newborns living in the capital city of Prague. MN analysis was performed in a group of 178 mothers and their newborns from two locations in the Czech Republic (86 from Prague and 92 from a regional city in Ceske Budejovice). The concentrations of benzo[a]pyrene (B[a]P), particulate matter <2.5µm (PM2.5) and benzene were measured by stationary monitoring. We observed differences in B[a]P concentration in Prague between years and between locations in winter season 2008/2009. The genomic frequency of translocations per 100 cells representing stable aberrations were 0.80±0.79 *vs.* 0.09±0.13 ($p<0.001$) for mothers *vs.* newborns. We observed 19.7% unstable aberrations *vs.* 80.3% stable aberrations in mothers and 64.3% *vs.* 35.7% in newborns, respectively. The frequencies of MN per 1000 binucleated cells were 8.35±3.06 *vs.* 6.47±2.35 ($p<0.001$) for mothers and 2.17±1.32 *vs.* 3.82±2.43 ($p<0.001$) for newborns from Prague and Ceske Budejovice, respectively. The results suggest that the different sensitivity of the study groups to various mixtures of carcinogenic pollutants could be affected by significant differences in lifestyle factors. Supported by Ministry of Education CR #2B06088.

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Micronuclei in Potentially Malignant Disorders and Oral Cancer. Feliciano LM, Minicucci EM, Marcondes JPC, Polizel DM, Dorth GO, Carboni TR, Salvadori DMF. Faculty of Medicine of Botucatu, São Paulo State University, Botucatu, SP, Brazil.

Micronucleus test in buccal exfoliated cells is a minimally invasive method and it is used to assess genomic instability and cancer risk. The risk of malignization of the oral potentially malignant disorders (PMD), as leukoplakia (OL) and lichen planus (LP) is discussed. The aim of this study was to evaluate the frequency of micronucleus (MN) in patients with OL (n=10), LP (n=16) and oral carcinomas (n=15). Exfoliated cells samples were collected from three oral mucosa sites: lesion site, around the lesion site, and opposite side to the lesion site. For control, a pool of oral mucosa cells was collected from healthy subjects (n=27). To check the frequency of MN, exfoliated oral mucosa cells were stained with Feulgen/fast green and analyzed in light microscopy under 400 x magnification. Statistical analysis was done using the Poisson distribution. Higher frequencies of MN were observed at the lesion site from patients with OL, LP and oral carcinomas than in healthy controls. In LP patients, the lesion site presented higher frequency of MN than the around and opposite side to the lesion site. Higher frequencies of MN were detected in opposite side to the lesion in OL patients than in controls. In conclusion, patients with OL, LP and oral carcinomas have increased frequency of micronucleus and, in some cases not restricted to the area of lesion.

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Investigating the Utility of the Muta™Mouse Transgenic Rodent Assay for Regulatory Decision-Making: A Multi-Endpoint Comparison of Several *In Vivo* False Negatives. Long AS[1,2], Lemieux CL[2], Dertinger S[3], White PA[1,2]. [1]Department of Biology, University of Ottawa, Ottawa, ON, Canada, [2]Mechanistic Studies Division, Environmental and Radiation Health Sciences Directorate, HECSB, Health Canada, Ottawa, ON, Canada, [3]Litron Laboratories, Rochester, NY, United States.

Test batteries to screen chemicals for mutagenic hazard include several endpoints regarded as effective for detecting mutagens and, by extension, mutagenic carcinogens. However, there is increasing recognition of response inconsistency. *In vitro* irrelevant positives elicit significant responses *in vitro* that cannot be confirmed *in vivo*. *In vivo* "false negatives" are rodent carcinogens that fail to induce a positive response *in vivo*. This study is investigating the ability of the Muta™Mouse lacZ mutation assay to resolve the latter. Three carcinogenic compounds that elicit positive responses *in vitro* for gene mutation, but are negative for cytogenetic damage *in vivo* in heamatopoetic cells, were selected for evaluation (*i.e.*, 4,4-methylenebis(N,N'-dimethylaniline) (*i.e.*, MBDA), 2,4-dinitrotoluene, nitrofen). Muta™Mouse specimens were exposed to 3 doses for 28 days *via* oral gavage. Following a 3-day fixation period, mice were sacrificed and tissues (*e.g.*, stomach, small intestine, bone marrow, liver) scored for lacZ mutant frequency. In addition, reticulocytes (RETs) and normochromatic erythrocytes (NCEs) were analyzed for Pig-a mutant phenotype and micronucleus (MN) frequency. Results to date show a statistically significant effect of MBDA on the frequency of MN-NCEs and Pig-a mutant phenotype in NCEs. LacZ mutants did not show a treatment effect for liver and bone marrow; several other tissues are being examined. Nitrofen, and 2,4-DNT exposures have been completed, and tissue analyses are underway. The results obtained will contribute to the validation of transgenic rodents mutation assays, and moreover, an appreciation of their ability to resolve conflicts between older *in vivo* endpoints and the results of cancer bioassays.

P72

Evaluation of Genotoxic Effect If Ionizing Radiation between Orthopedic Surgeons Exposed by Micronucleus Assay. Sierra BY[1], Groot H[2], Plazas MC[1], Eslava-Schmalbach J[1]. [1]Universidad Nacional de Colombia, Bogotá, Colombia, [2]Universidad de los Andes, Bogotá, Colombia.

Objective: To evaluate the genotoxic effect of ionizing radiation over the DNA of orthopedic surgeons exposed to low doses as a consequence of the use of fluoroscopic machine, compared with a non-exposed group of people from general population. Methods. Double match cohort by age and gender, 31 exposed orthopedic surgeons and 31 non-exposed people selected from a general community. Follow up was performed over six months, evaluating the personal dosimetry and genotoxic effect through micronucleus assay with cytokinesis block, at the beginning and at the end of the observation period. Non-parametric statistics for matched data were used. Results. Genotoxic effect was observed at the beginning of the research over exposed group, six months later, the Micronucleus frequencies were similar to the one found over the non-exposed individuals, (Me 8, IC95%6-12 *vs.* Me 4 IC95%2,4-8, p=<0,05). The higher values of micronúcleos frequencies between exposed ones were correlated with the exposition time over years, first month dosimetry and cumulative dose. The Micronucleus assay results over the non-exposed group did not show differences between the two samples taken. There was no correlation between dosimetry measures and micronucleus assay frequency. Conclusions. It is possible to find DNA alterations through a micronucleus assay as a consequence of exposition to low radiation doses. The influence of the observation effect was important over the obtained data, which proves that reducing the exposition and following the precautions, the dose and genotóxic effect are reduced. It is important to improve the measurements of radioprotection for exposed personnel.

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Simultaneous Measurements of DNA Adducts, Pig-a and lacZ Mutations, and Micronuclei-Induced by 3 PAHs. Lemieux CL[1], Arlt VM[2], Dertinger SD[3], Phillips DH[2], White PA[1]. [1]Mechanistic Studies Division, Environmental Health Sciences and Research Bureau, Health Canada, Ottawa, ON, Canada, [2]Section of Molecular Carcinogenesis, Institute of Cancer Research, Sutton, Surrey, United Kingdom, [3]Litron Laboratories, Rochester, NY, United States.

This study compared the responses obtained with the Pig-a mutation assay to those of the lacZ mutation assay for 3 polycyclic aromatic hydrocarbons (PAHs): benzo[a]pyrene (BaP, IARC Group 1), dibenz[a,h]anthracene (DBahA, IARC Group 2A) and benz[a]anthracene (BaA, IARC Group 2B). Adult male Muta^{TM}Mouse were exposed to each of the PAHs for 28-days *via* oral gavage, and measurements of DNA adducts, lacZ mutations, mutant Pig-a phenotype erythrocytes (RBC^{CD24-}) and reticulocytes (RET^{CD24-}), and micronucleated reticulocytes (MN-RET) and normochromatic erythrocytes (MN-NCE) were obtained. Dose-dependent increases in DNA adducts were observed, confirming metabolic activation and delivery to target tissues. All 3 PAHs induced significant dose-dependent increases in lacZ mutations, DBahA and BaP induced significant increases in %MN-RET and %MN-NCE, and BaA and BaP induced significant increases in RET^{CD24-} and RBC $^{CD24-}$. PAH doubling dose (DD, dose required to induce a 2-fold increase above spontaneous) for induction of lacZ mutants showed the following trend: BaP < DBahA < BaA. Moreover, for the PAHs that yielded positive responses with the Pig-a assay (BaP, BaA), the Pig-a DD was not significantly different from the lacZ DD for bone marrow. The DD for %MN was also consistently higher than that derived from the two mutation assays. The results contribute to the validation of the Pig-a assay, and support the use of DD as a response metric for comparison across endpoints. This study also demonstrates the feasibility of integrating multiple measurements of DNA damage into a subchronic study, thus reducing the number of animals required for routine toxicity testing.

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***In Vivo* Multi-Endpoint Investigation of Chlorambucil Genotoxicity: Pig-a Mutation, Micronucleus, and Comet Assays.** Torous D[1], Vasquez M[2], Phonethepswath S[1], Weller P[1], Avlasevich S[1], Mareness J[1], Bemis J[1], Sivers C[2], Dewhurst N[2], MacGregor J[3], Dertinger S[1]. [1]Litron Laboratories, Rochester, NY, United States, [2]Helix3, Morrisville, NC, United States, [3]Toxicology Consulting Services, Arnold, MD, United States.

Integration of endpoints to reduce, refine and replace the use of animals is a major theme in regulatory toxicity testing, including genotoxicity. In these studies of chlorambucil, a nitrogen mustard-type antineoplastic drug, three blood-based endpoints were evaluated: flow cytometric measurement of Pig-a mutant phenotype cells and micronucleated reticulocytes (MN-RET), and Comet. Male Sprague Dawley rats were treated for 3 or 28 consecutive days with several doses of chlorambucil and Pig-a mutant frequencies were determined over a period of weeks. Blood MN-RET were measured on Day 4 (of both studies) and Day 29 of the subchronic study. Blood was obtained for Comet analysis 4 hours after treatment on Days 3 and 28. Chlorambucil induced robust dose-dependent Pig-a mutation and MN-RET responses in both study designs. For Comet, statistically significant increases in Olive Tail Moment (OTM) were observed in both studies, but a mechanism involving cytotoxicity could not be ruled out since low molecular weight DNA diffusion or histopathology data were not available. A follow-up acellular Comet assay was performed and showed a clear and significant dose-dependent increase in DNA migration attributed to DNA damage (and not cytotoxicity) given the design of the acellular assay. Interestingly, the acellular data do not suggest that protein-DNA or DNA-DNA cross-linking were a major contributor to the positive findings. Taken together, results from these studies suggest that integration is both possible and desirable, but results should be interpreted in the context of cytotoxicity data, especially if the study design is subchronic or chronic in nature.

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Integration of Pig-A, Micronucleus and Comet Assay Endpoints in 28-Day Rodent Toxicity Studies with 7,12-dimethylbenz(a)anthracene (DMBA) and diethylnitrosamine (DEN). Stankowski, Jr. LF[1], Krsmanovic B[1], Bruce S[1], Kelley T[1], Paranjpel M[1], Szabol K[1], Springer S[1], Sly J[1], Klug-LaForce M[1], Arevalo M[1], Atta-Safoh S[1], Debelie F[1], Sareen P[1], Dertinger S[2], Shi J[1]. [1]BioReliance Corporation, Rockville, MD, United States, [2]Litron Laboratories, Rochester, NY, United States.

As part of a multi-lab validation, we examined induction of Pig-a mutant red blood cells (RBCs) and reticulocytes (RETs) by flow cytometry (FCM) during 28-day subchronic studies in male Sprague-Dawley rats treated with 2.5, 5 and 10 mg/kg/day DMBA, or 5, 10, 20 and 35 mg/kg/day DEN. The same animals were analyzed for micronucleated RETs (mnRETs) in peripheral blood by FCM, and DNA damage in liver *via* Comet assay. Also analyzed were Comet response in peripheral blood (DEN-treated animals), or micronucleated polychromatic erythrocytes (mnPCEs) in bone marrow (DMBA-treated animals, by manual microscopy). DMBA induced dose-related increases in Pig-a mutant RBCs and RETs (Days 15 and 29), mnRETs (Days 4 and 29), and mnPCEs (Day 29), but no increase in Comet response was observed in liver at doses up to 10 mg/kg/day, which appeared to be below the MTD (upon re-testing up to 200 mg/kg/day for 3 days in a follow-up acute study, DMBA induced a positive Comet response in liver, but was negative in peripheral blood). In contrast, DEN was negative for induction of Pig-a mutant RBCs and RETs (Days 15 and 29) and mnRETs (Days 4 and 29), but induced dose-dependent increases in Comet response in liver and blood (Day 29), at doses up to 10 mg/kg/day (higher dose groups were terminated early due to excessive toxicity/mortality). These results emphasize the extreme care that must be taken in dose and endpoint selection when incorporating genotoxicity endpoints into routine toxicity studies.

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Integration of Pig-A, Micronucleus, Chromosome Aberration and Comet Assay Endpoints in Rodent Toxicity Studies with 4-nitroquinoline-1-oxide (4nqo): Effect of Dose Fractionation on Genotoxic Responses. Stankowski, Jr. LF[1], Roberts DJ[2], Chen H[3], Lawlor T[3], McKeon M[3], Murli H[3], Thakur A[3], Xu Y[3]. [1]BioReliance Corporation, Rockville, MD, United States, [2]Bristol-Myers Squibb, New Brunswick, NJ, United States, [3]Covance Laboratories, Inc., Vienna, VA, United States.

As part of a multi-lab validation, we examined induction of Pig-a mutant red blood cells (RBCs) and reticulocytes (RETs) by flow cytometry (FCM) during a 28-day subchronic study in male Sprague-Dawley rats using 4NQO. Animals also were analyzed for: micronucleated RETs (mnRETs) by FCM; DNA damage in blood, liver, and stomach by the Comet assay; and chromosome aberrations (CAbs) in peripheral blood lymphocytes (PBLs). Dose-and time-related increases were observed in Pig-a mutant RBCs and RETs (Days 15, 29), as well as mnRETs (Day 29, but not 4). No increases were observed in PBL CAbs (Days 4, 29), or DNA damage in liver (Days 15, 29), stomach (Day 29), or PBLs (Days 1, 15, 29). A follow-up study was performed at the same cumulative doses given in 1 or 3 daily doses (the latter as in a typical Comet/micronucleus combo assay). Dose-related increases were observed for Pig-a mutant RBCs and RETs (15 and 29 days after last dose), mnRETs (3 days after last dose), and Comet responses in PBLs, liver and stomach (3 hours after last dose). Except for Pig-a mutant RETs and RBCs, all responses decreased at the later timepoints. Dose-dependent increases in micronucleated bone marrow polychromatic erythrocytes also were observed 3 hours after the last of 3 doses, but no increase in CAb was observed in PBLs, possibly due to technical issues. These results emphasize the extreme care that must be taken in dose and endpoint selection when incorporating genotoxicity endpoints into routine toxicity studies as recommended in ICH S2(R1).

P77

A Japanese Collaborative Study on Rat Pig-a Assay; Report on a Transferability of the Assay Method and Interlaboratory Difference. Kimoto T[1], Horibata K[2], Muto S[3], Sanada H[4], Hashimoto K[5], Ito S[5], Uno Y[3], Honma M[2]. [1]Teijin Pharma Limited, Tokyo, Japan, [2]National Institute of Health Sciences, Tokyo, Japan, [3]Mitsubishi Tanabe Pharma Corporation, Chiba, Japan, [4]Kaken Pharmaceutical Co., LTD., Shizuoka, Japan, [5]Daiichi Sankyo Co., LTD, Shizuoka, Japan.

The Pig-a assay in peripheral blood (PB) is anticipated as a useful tool for evaluating *in vivo* mutagenicity. The goal of this collaborative study is to establish an optimal Pig-a assay protocol for integration of this study to other toxicology studies. As a first step, two trials have been conducted to confirm the operability in participating laboratories. We employed the rat Pig-a assay using an antibody to erythroid marker (HIS49), to identify erythrocytes in PB. First we discussed how we could set an appropriate region in cytograms to detect CD59-negative erythrocytes. After that, 6 or 7-week-old CD (SD) rats were administrated a single oral dose of 0, 10 and 40 mg/kg ENU. Four laboratories conducted animal dosing and blood sampling at 0, 2 and 4 weeks after the treatment. The Pig-a assay was performed in five laboratories, one of which received the blood samples from another laboratory. The preliminary common region setting made the spontaneous frequency of CD59-negative erythrocytes less than 10×10^{-6} in all laboratories. In addition, they have shown similar dose-dependent positive response to ENU, while the single dose of 10 mg/kg ENU showed weak response. These results indicate that the Pig-a assay is easy to transfer for sharing the assay method and the interlaboratory difference among four laboratories in the response to ENU is very small. This work was supported by Japan Health Sciences Foundation, Grant Number: KHB1006.

P78

Interlaboratory Pig-a Mutation Assay Collaborative Trial Update: Stage IV. Bemis JC[1], Torous DK[1], Weller PC[1], Phonethepswath S[1], Avlasevich S[1], Bryce SM[1], Mereness J[1], MacGregor JT[2], Dertinger SD[1]. [1]Litron Laboratories, Rochester, NY, United States, [2]Toxicology Consulting Services, Arnold, MD, United States.

An international collaborative trial was established to systematically investigate the merits and limitations of a rat *in vivo* Pig-a gene mutation assay. In this assay flow cytometry is used to quantify circulating mutant phenotype reticulocytes (RET^{CD59-}) and erythrocytes (RBC^{CD59-}). Initial interlaboratory data have been informative, but the time required for those flow cytometric analyses (~20 minutes) limited the number of cells per sample that could be interrogated for the mutant phenotype, thus negatively impacting sensitivity. It was therefore desirable to establish a higher throughput scoring approach before expanding the trial to include additional mutagens as well as non-genotoxicants ("Stage IV"). An immunomagnetic column separation method that dramatically increased analysis rates was developed and has been used to study male Sprague Dawley rats treated for 3 or 28 consecutive days with several doses of 1,3-propane sultone, melphalan, thiotepa, chlorambucil, or 2-acetylaminofluorene. Pig-a mutant frequencies were determined over a period of several weeks and supplemented with peripheral blood micronucleated reticulocyte counts. Each mutagen increased Pig-a mutation and micronucleated reticulocyte frequencies in both study designs. While the greatest inductions of micronuclei were observed in 3-day studies, the highest Pig-a responses occurred following 28-days of treatment. By using the immunomagnetic separation technique, Pig-a measurements were acquired in approximately one third the time required for the original method, while the number of erythrocyte and reticulocyte equivalents interrogated per sample were increased by factors of 100 and 10, respectively. The data strongly support the value of using the immunomagnetic separation technique for enumerating Pig-a mutation frequencies.

P79

Pig-a Mutation Analysis of Benzo[a]Pyrene Treated Rats. Bhalli JA[1], Vohr HW[2], Heflich RH[1]. [1]National Center for Toxicological Research (NCTR), U.S. Food and Drug Administration (FDA), Jefferson, AR, United States, [2]Toxicology, Bayer Health Care AG, Wuppertal, Germany.

Benzo[a]pyrene (BaP) is a mutagenic and carcinogenic polycyclic aromatic hydrocarbon that is found in the environment as a product of incomplete combustion. It was one of the first compounds demonstrated to be converted by mammalian cell metabolism to products that bind DNA, and subsequently produce mutations. National Center for Toxicological Research-US FDA (FDA-NCTR) and Bayer Schering Pharma, Germany (Bayer) conducted a Stage III trial measuring the genotoxicity of BaP in Fischer 344 (FDA-NCTR) and Han Wistar rats (Bayer) as part of an international validation study of the Pig-a assay. Groups of five 6-7-week-old male rats were given 28 daily doses of 0 (sesame oil vehicle), 37.5, 75, or 150 mg/kg BaP. Pig-a mutant frequencies were determined on Days -1, 15, 29 and 56 in red blood cells (RBCCD59-) and reticulocytes (RETCD59-), while micronucleus frequencies were measured as %MN-RETs on Days 4 and 29. RBCCD59- and RETCD59- frequencies increased in a dose- and time-dependent manner, producing significant increases by Day 29 in both rat models. In general, stronger responses were found for RETCD59- than RBCCD59-, and the responses in F344 rats were stronger than in Han Wistar rats. An increase in %MN-RET frequency was observed at Days 4 and 29, with the responses being greater in F344 than Han Wistar rats. The results of the study were consistent with those of the reference laboratory (Litron Laboratories). The findings indicate that the Pig-a assay is reproducible, transferable, and shows promise for integrating a gene mutation endpoint into 28-day repeat-dose studies.

P80

New Assays for Measuring Pig-a Mutant Frequency in Rat Peripheral Blood Reticulocytes (the PIGRET assay) and Bone Marrow Erythroids. Kimoto T[1], Chikura S[1], Suzuki K[1], Kobayashi XM[1], Itano Y[1], Horibata K[2], Honma M[2], Dobrovolsky VN[3], Heflich RH[3], Miura D[1], Kasahara Y[1]. [1]Teijin Pharma Limited, Tokyo, Japan, [2]National Institute of Health Sciences Japan, Tokyo, Japan, [3]U.S. Food and Drug Administration/National Center for Toxicological Research, Jefferson, AR, United States.

The Pig-a assay is currently being investigated as a tool for assessing mutagenicity *in vivo*. Several approaches have been reported for measuring Pig-a mutant frequency in rat peripheral red blood cells (RBCs) and reticulocytes (RETs); however, the RET assays suffer from low through-put, and neither RETs nor RBCs have nuclei, making the characterization of mutations difficult. We have developed methods to increase throughput of the RET assay and measure mutants in nucleated bone marrow erythroids (BMEs). These assays use a combination of antibodies to CD71 and the erythroid marker HIS49. The new Pig-a assay for RETs, the PIGRET assay, employs magnetic enrichment of RETs and enabled us to interrogate more than 1×10^6 CD71-positive RETs for Pig-a mutation by flow cytometry in 5-8 min. The PIGRET assay detected apparent increases in RET Pig-a mutant frequency one week after a single treatment with 40 mg/kg ENU (from 1.2×10^{-6} in control animals to 124.8×10^{-6} in ENU-treated animals), while the total RBC Pig-a mutant frequency in treated animals was still quite low (13.2×10^{-6}). In addition, the Pig-a mutant frequency data for BMEs was highly correlated with that determined by PIGRET. Methods like PIGRET and the BME assay, that measure mutation in early-responding cell populations, may be useful for integrating the Pig-a assay into *in vivo* toxicology assays. In addition, it should be possible to sequence Pig-a mutants induced in the BME assay, thus confirming the mutational basis of the assay. This work was supported by the Japan Health Sciences Foundation, Grant Number: KHB1006.

P81

Evaluation of the Pig-a Mutation Assay at Janssen R&D Using the Direct Acting Alkylation Agent N-Ethyl-N-Nitrosourea As Model Compound. van der Leede BM[1], Van Doninck T[1], Schuermans A[1], Dertinger SD[2], Van Gompel J[1]. [1]Janssen Research & Development, a division of Janssen Pharmaceutica NV, Beerse, Belgium, [2]Litron Laboratories, Rochester, NY, United States.

The Pig-a gene codes for an enzyme that is required for the synthesis of glycosylphosphatidyl inositol anchors that attach proteins (*i.e.* CD59) to the cell surface. Recently an *in vivo* mutation assay has been developed based on the enumeration of CD59-negative rat erythrocytes serving as a phenotypic marker of Pig-a gene mutation. In an ongoing international collaborative trial (coordinated by Litron Laboratories) more than 20 laboratories participate to evaluate the potential of the assay for its use in safety assessment of pharmaceuticals. As initial contribution to the collaborative trial, we examined the induction of CD59-negative erythrocytes in male Sprague-Dawley rats treated with N-ethyl-N-nitrosourea (ENU) at 0, 20 and 40 mg/kg/day for 3 consecutive days *via* oral gavage (n=5 per group). Blood samples collected on Days-1, 5, 30 and 45 were processed and analysed by flow cytometry to determine the % reticulocytes, frequency of CD59-negative reticulocytes (RET^{CD59-}) and frequency of CD59-negative erythrocytes (RBC^{CD59-}). Dose-related increases in the frequency of RET^{CD59-} and RBC^{CD59-} were observed on Day 15. The increases in RET^{CD59-} frequency appeared to be maximal on Day 15, whereas the RBC^{CD59-} frequency increased gradual over time with peak responses on Day 45. Our data correlate well with data from Litron Laboratories. As second contribution to the collaborative trial, we demonstrated that by analysis of technical replicates the assay is highly reproducible, as the experimental coefficients of variation approached theoretical values. In conclusion, our studies indicate that the Pig-a mutation assay is easily portable between laboratories and generates highly reproducible data.

P82

Investigation of Methodical Bias in the *In Vivo* Comet Assay. Beevers C, Henderson D, Whitwell J, Lillford L. Covance Laboratories Limited, Harrogate, United Kingdom.

In 2010 and 2011 a series of articles and comments published in Mutagenesis questioned the relevance of low level, monotonic increases in DNA damage as detected in a small number of *in vivo* alkaline comet assays, performed at Covance Laboratories Limited, Harrogate, UK. Whilst contributions from Cederberg *et al.* (1, 2), Lillford *et al.* (3) and D Lovell (4) concentrated on the roles of statistics and biological relevance in the interpretation of Comet data, Struwe *et al.* (5) focused on the causes on monotonic increases in Comet data and suggested a methodical bias was responsible for the observed monotonic increases. We investigated the possibility of methodical bias by analysing a more comprehensive set of data generated at Covance over the previous 18 months. This analysis focused on the fold-increase in treated groups, compared to the concurrent vehicle control, the actual process order used to isolate single cells from treated animals and all other supporting data available for interpretation of the Comet data. In addition, we examined liver comet data from 24 untreated animals, processed in a strict pre-determined order, for the presence of any monotonic increase in DNA damage. Our investigations suggest our process methods do not cause bias in Comet data and that interpretation of Comet data requires an evaluation of all the data available; statistical evaluation alone is insufficient. 1) Cederberg *et al. Mutagenesis*. 2010 **25** p133-8. 2) Cederberg *et al. Mutagenesis*. 2010 **25** p420-430. 3) Lillford *et al. Mutagenesis*. 2010 **25** p427-428. 4) Lovell. *Mutagenesis*. 2010 **25** p443-446. 5) Struwe *et al. Mutagenesis*. 2011 **26** p473-475.

P83

Genotoxicity of Intravenous Anesthesia with Propofol in Peripheral Lymphocytes of Patients Undergoing Elective Surgery. Freire CMM[1], Braz MG[1], Braz LG[2], Camargo EA[1], Salvadori DMF[1]. [1]Departament of Pathology, Botucatu, SP, Brazil, [2]Departament of Anesthesiology, Botucatu, SP, Brazil.

DNA is continuously exposed to a variety of biological, chemical and physical agents, which may alter its structure, modifying its function. Among the exogenous genotoxic compounds, anesthetics used in general anesthesia have drawn considerable attention because of their possible secondary effects. Nevertheless, little is known about the *in vivo* genotoxicity of the anesthetic propofol (PF), one of the most used intravenous anesthetic in the world. The aim of the study was to investigate the genotoxic potential of PF in peripheral blood lymphocytes collected from patients submitted to otorhinological surgery. Eight patients, of both sexes, classified by the American Society of Anesthesiologists as physical status I (healthy patient, without disease other than surgical abnormality, and without systemic disturbances), and scheduled for non-invasive surgery were recruited. Peripheral blood samples were collected at two time points: before anesthesia and surgery (T_1-control) and 2 h after the beginning of anesthesia (T_2). Lymphocytes were isolated, and the comet assay was conducted to detect oxidative DNA damage (8-oxoguanine), which is recognized by the enzyme OGG1. Tail intensity was considered to estimate DNA damage, which was evaluated using the Comet Assay IV software. Results showed no statistically significant difference between the two sampling times. In conclusion, the anesthesia with PF did not induce oxidative damage in DNA in peripheral lymphocytes of patients undergoing elective surgery, suggesting absence of genotoxicity of this anesthetic. Financial Support: FAPESP and CNPq.

P84

Effect of Gemcitabine on DNA Structure and Gene Expression in Urinary Bladder Carcinoma Cell Lines. Camargo EA, da Silva GN, Gobette CP, Marcondes JPC, Salvadori DMF. Botucatu Medical School, Botucatu, SP, Brazil.

Bladder cancer is the fourth most common malignancy in the Western world, with the transitional cell carcinoma comprising about 90% of urinary tumors. The antineoplastic drug gemcitabine, commonly used to treat bladder cancers, is a deoxycytidine analog that incorporates itself into DNA, causing replication blockage. The present study was designed to evaluate the effects of gemcitabine on cell proliferation and on expression of genes related to cell cycle in two urinary bladder carcinoma cell lines (5637 and T24) with mutated TP53. Cell viability assays, cell proliferation and quantitative polymerase chain reaction - reverse transcriptase (qPCR-RT) were used to investigate the effects of gemcitabine at concentrations of 0.78 μM, 1.56 μM and 3.12 μM. Data showed decreased nuclear division index (NDI) in the two cell lines after treatments with gemcitabine. T24 cell viability was significantly lower after treatment with gemcitabine (3.12 μM), when compared to control (86.23%). Data obtained in the real-time quantitative RT-PCR showed that the genes related to cell cycle arrest (CDK2/CDKN1A), response to DNA damage (BRCA1, BRCA2, TP53) and kinase activity (GADD45A) were highly expressed in line cell 5637 and T24 after treatment with gemcitabine. Gemcitabine presented *in vitro* cytostatic effect on T24 and 5637 urinary bladder carcinoma cells, modulating genes related to cell cycle arrest. FAPESP and CNPq.

P85

DNA Reactivity Dose-Response Curves Using the Acellular Comet Assay. Vasquez MZ, Dewhurst NE, Sivers CL. Helix3 Inc., Morrisville, NC, United States.

The high occurrence of positive results in the current battery of *in vitro* genotoxicity tests and their poor concordance with *in vivo* carcinogenicity results have been a significant source of concern in safety testing. Among the suspected causes of the high frequency of positive *in vitro* results are a dependency of most assays on cellular division and the confounding effects of cytotoxicity, repair, cell line differences and multiple modes of action. To supplement the specificity of *in vitro* testing and minimize the consequentially required *in vivo* tests, the acellular comet assay can be used to provide DNA reactivity dose-response curve data using minimal test compound, time and expense. More sensitive and specific than the classic *in vitro* comet assay, the acellular comet assay avoids those factors that confound live cell assays while providing a fast method for determining DNA reactivity and elucidating the possible mode of action (MoA) of a test compound. To demonstrate how the acellular comet assay may be used effectively, we present DNA reactivity dose-response curves generated for multiple compounds each with a different mode of action and/or genotoxicity profile.

P86

Genotoxicity Safety Testing in Reconstructed 3-D Human Epidermal Skin Models Using the Comet Assay. Downs TR[1], Reus A[2], Reisinger K[3], Krul C[2], Pfuhler S[1]. [1]Procter & Gamble Co, Cincinnati, OH, United States, [2]TNO Quality of Life, Zeist, Netherlands, [3]Henkel AG & Co KgaA, Düsseldorf, Germany.

Reconstructed 3-D human epidermal skin models are being increasingly used for safety testing of chemicals. Based on EpiDerm™ tissues, we developed a 3-D skin assay where test compounds are topically exposed for 3h followed by cell isolation and assessment of DNA damage using the Comet assay. Inter-laboratory reproducibility of the 3-D skin comet assay was initially demonstrated for MMS and 4NQO and results showed good concordance among 4 different labs and with *in vivo* data. In the current project phase, intra- and inter-laboratory reproducibility was investigated with 5 coded compounds tested at 3 different laboratories. For compounds 2 and 3, all labs found a dose-related increase in DNA damage in every experiment. For compound 4, the overall result from all labs showed a smaller, but significant genotoxic response. For compound 1, an increase compared to solvent controls was observed only in one lab. However, the response was not dose-related so compound 1 was judged negative overall, as was compound 5, which was the only compound showing clear cytotoxic effects. For compound 5, significant DNA damage generally occurred only at doses that showed substantial cytotoxicity (>30% cell loss), and the overall response was comparable in all labs despite some differences in doses tested. The results of the collaborative study for the coded compounds were generally reproducible among the laboratories involved and intra-lab reproducibility was also good. These data indicate that the comet assay in reconstructed 3-D skin models is a relevant model for the safety assessment of chemicals with dermal exposure.

P87

Effect of Four Antioxidants on DNA Damage Measured by the Comet Assay. Sharma S, Naravaneni R, Bhaumick D, Mehta RD. PBR Laboratories Inc., Edmonton, AB, Canada.

Our body cells produce free radicals which can cause oxidative DNA damage. Antioxidants are free radical scavengers, which can prevent or slow the oxidative damage. In the present study, we investigated the effect of four antioxidants (Ascorbic acid, Glutathione, Resveratrol and Trolox (derivative of vitamin E)) on DNA damage in human leukocytes using the Comet assay. Blood samples were treated with various concentrations (90, 180 and 360 μM) of antioxidants for 1 hr at 37°C and analyzed with the comet assay. Using the VisCOMET software, the degree of damage was evaluated as the Tail DNA%. Our results revealed that, there was a significant decrease in DNA damage in the samples treated with Ascorbic acid at a lower concentration (90 μM), where as there was a significant increase in DNA damage at higher concentrations >180 μM. Glutathione treatment resulted in decreasing a DNA damage at only the highest dose (360 μM). There was a dose related DNA damage increase observed in cells treated with Resveratrol. The treatment of Trolox showed a significant increase in DNA damage at the highest dose (360 μM) tested. Based on our results we conclude that the effect of antioxidants on DNA damage is variable and concentration dependent.

P88

Evaluation of Occupational Pesticide Exposure in Rice Farmers. Olaya LF[1], Narváez DM[1], Díaz S[2], Briceño L[3], Varona M[2], Grot de Restrepo H[1]. [1]Universidad de los Andes, Bogotá, Colombia, [2]Instituto Nacional de Salud, Bogotá, Colombia, [3]Universidad del Rosario, Bogotá, Colombia.

The use of pesticides is an essential part of agricultural practices in many countries. However, its overuse or misuse can lead to serious consequences for public health and for the environment. Rice ranks as first in terms of its economic value in Colombia, which is also the second largest producer in Latin America and the Caribbean. The aim of this study was to determine DNA damage and the working conditions of rice farmers exposed to pesticides. A transversal descriptive study was carry out in a population occupationally exposed to pesticides in a rice agricultural region in Colombia. Demographic and occupational information was taken into account to describe the population. The levels of organophosphates, carbamates, dithiocarbamates and organochlorines, were determined in human fluids as a biomarker of exposure. The comet assay (pH>13) was conducted to established DNA damage as biomarker of effect. Results showed mainly, levels of organochlorines in blood. On the other hand, we found significant differences between in the use and not use of pesticides within the farmers in terms of the percentage of DNA in comet tail. In conclusion, there is an effect of pesticides use in the DNA of rice farmers.

P89

Evaluation of Cigarette Smoke-Induced Cell Transformation Using Three *In Vitro* Cell Transformation Assays. Fowler KW[1], Fields WR[1], Nordskog BK[1], Potts RJ[1], Bombick BR[1], Pant K[2]. [1]R. J. Reynolds Tobacco, Winston-Salem, NC, United States, [2]BioReliance Corporation, Rockville, MD, United States.

Cell transformation assays (CTA) detect phenotypic changes associated with neoplastic transformation and available evidence suggests *in vitro* cell transformation may simulate early components of the multistage process of carcinogenesis. Moreover, CTAs may aid in identifying potentially non-genotoxic carcinogens which may not be detected by conventional genotoxicity assays. We have tested whether total particulate matter (TPM) and cigarette smoke condensate (CSC) generated from Kentucky reference 3R4F cigarettes induce morphological transformation using three versions of the CTA: the Syrian Hamster Embyro (SHE) cell transformation assay using 24-hour and 7-day exposure protocols, the SHE assay using initiator/promotor protocols, and initiator/promotor assays using B-has 42 cells (v-Ha-ras-transfected Balb/c 3T3 cells). TPM and CSC were positive in the SHE 7-day exposure with 12.5- and 9-fold increases, respectively, in the number of morphologically transformed colonies as compared to solvent controls; CSC was positive (2.7-fold increase) while TPM was equivocal in the SHE 24-hour exposure assay. TPM and CSC induced positive responses in the SHE initiator and promotor assays with approximately 3-fold increases in the number of morphologically transformed colonies. In the Bhas-42 initiator assay, TPM produced a positive response (3.5-fold increase) while CSC was equivocal; however, in the B-has 42 promotor assay both TPM and CSC were positive, inducing approximately a 12-fold increase in morphologically transformed foci. Among the CTA models investigated, the B-has 42 promotor assay provided the most linear response coupled with a robust increase in transformed foci across the treatment range and may therefore be a useful technique to evaluate cigarette samples.

P90

BioReliance *In Vivo* Comet Assay Historical Vehicle and Positive Control Data. Pant K, Bruce SW, Klug Laforce M, Sly JE, Atta-Safoh S, Springer S, Kelley T, Debelie F, Arevalo M, Krsmanovic L. BioReliance, Rockville, MD, United States.

In recent years the Comet assay has become very popular as a second *in vivo* assay and as an integrated assay with the micro nucleus or long term mammalian toxicology assays. The biggest advantage of the Comet assay is that it can be performed in most organs as long as a single cell suspension can be prepared. To be able to properly interpret the result of a Comet assay, it is extremely important to have historical vehicle and positive control data for all the different organs. BioReliance has a robust historical vehicle and positive control database for a variety of tissues in mice and rats. We routinely perform Comet assay on the following organs in mice - (liver, kidney, spleen, bone marrow, stomach and skin), rat -(blood, colon, duodenum, jejunum, kidney, liver, lung, stomach, uterus, bone marrow and spleen). Different organs require different single cell suspension preparations such as mincing for liver and spleen, scraping for glandular stomach, duodenum, jejunum *etc.* and enzymatic digestion method is used for skin. The historical control data is also important for different routes of test article administration. Data will be presented and the technical aspects of cell and slide preparation, electrophoresis parameters and scoring aspects will be discussed for the successful conduct of the Comet assay. Recommendations are based on the JaCVAM (Japanese Center for the Validation of the Alternative methods) protocol version 14.2.

P91

Cell Transformation Activity of Cigarette Smoke Condensate in Bhas 42 Assay. Pant K[1], Bruce SW[1], Gairola CG[2]. [1]BioReliance, Rockville, MD, United States, [2]University of Kentucky, Lexington, KY, United States.

Cell transformation assays have been commonly used to predict chemical carcinogenicity. Using v-Ha-ras transfected BALB/c3T3 (Bhas42) cells, Sasaki and coworkers (*Jpn. J. Cancer Res.* 79 (1988) and Basic Life Sci. 52 (1990)) have validated a sensitive short-term assay that measures tumor initiating and promoting activities of chemicals. The present study determined the cell transformation activity of cigarette smoke condensate (CSC) in this Bhas42 cell assay. DMSO and water extracts of CSC (CSC-D & CSC-W, respectively) from the University of Kentucky reference cigarettes were tested in the initiator or promoter assay formats. The results of the initiator assay showed up to 3.5- fold increases in transformed foci at 40µg/ml of CSC-D but none by CSC-W. In contrast, robust dose responses (up to 14-fold increase) was observed in the promoter assay for CSC-D between 5µg to 40µg/ml and for CSC-W between 20µg to 40µg/ml. Pre- and co-incubation of Bhas42 cells with selenium significantly reduced initiator activity of CSC suggesting a role of oxidative stress in CSC-induced cell transformation. Co-treatment of cells with a sub-toxic dose of arsenate significantly enhanced cell transformation activity of CSC-D in the promoter assay. The results suggest i) the presence of both water soluble and insoluble tumor promoters in CSC, ii) a role of oxidative stress in CSC-induced cell transformation iii) a potential interaction of smoking with environmental metal pollutants in influencing neoplastic changes, and iv) a potential for application of Bhas-42 assay in initiator/promoter activity assessment of CSCs from different cigarettes.

P92

Evaluating Human Biomarkers of Exposure to Wood Smoke Resulting from Traditional Temazcal Use. Long AS[1], Lemieux CL[1], Shaikh N[2], Yousefi P[2], Mercado IR[2], Lam N[2], White PA[1], Smith K[2], Holland N[2]. [1]Mechanistic Studies Division, EHSRB, ERHSD, HECSB, Health Canada, Ottawa, ON, Canada, [2]School of Public Health, University of California, Berkeley, CA, United States.

Traditional temazcals (*i.e.*, sweat lodges) are commonly used by native populations in Central America. Temazcals are often heated *via* biomass combustion, and exposure to particulate combustion emissions has been associated with numerous adverse health effects including cancer. Urinary mutagenicity is a known biomarker of human exposure to combustion emissions, and this study evaluates urinary mutagenicity in regular temazcal users alongside other measures of exposure. The study subjects are indigenous Mayan families from Guatemala who regularly use temazcals, as well as control individuals from the same population. Urine samples collected before and after temazcal exposure were enzymatically hydrolyzed and urinary metabolites were concentrated by solid-phase extraction. Mutagenic potency (MP) of concentrated extracts was assessed using the Ames/*Salmonella* assay. Exhaled carbon monoxide (ppm) and blood CO levels (%COHb) were also measured, both of which were significantly increased following exposure ($p<0.0001$). A trend towards increasing mutagenicity was observed following temazcal exposure (2.1-fold increase over controls). Blood and exhaled CO levels were significantly correlated with the MP of the urine concentrates ($p=0.001$; $p=0.002$), and the MP of urine concentrates from exposed individuals was significantly correlated with time spent in the temazcal ($p<0.05$). Thus, the use of temazcals results in the production and urinary excretion of mutagenic metabolites. Moreover, the extent of temazcal use leads to an increase in urinary MP, and urinary MP is empirically related to other measures of exposure; confirming that urinary mutagenicity can be used as an effective biomarker of exposure to mutagenic combustion emissions in wood smoke in traditional temazcals.

P93

Relationship between Propofol or Sevoflurane Exposure and *IL-6* and *IL6-R* Gene Polymorphisms in Patients Undergoing Surgery. Padovani JL, da Silva GN, Braz MG, Monteiro MS, Marcondes JPC, Braz JRC, Salvadori DMF. Department of Pathology, Botucatu Medical School, UNESP, São Paulo State University, Botucatu, SP, Brazil.

Inflammatory processes, usually observed in patients after surgical procedures, can lead to immunosuppression. The inflammatory cytokine release seems to be different depending on the type of surgery and anesthetic used. Furthermore, interleukin (IL) genes polymorphisms can also influence the release of inflammatory mediators. Sevoflurane is the most commonly used inhaled anesthetic, and propofol is a widely used intravenous compound in general anesthesia. The aim of this study was to evaluate possible interference of interleukin-6 -174 G/C (rs1800795) and -572 G/C (rs1800796), and interleukin-6 receptor (rs8192284) gene variants on the inflammatory cytokine IL-6 release in patients submitted to surgery under general anesthesia with propofol or sevoflurane. We evaluated 32 young adult patients undergoing non-invasive surgery, randomly allocated in two groups: those under general anesthesia with sevoflurane (n = 17) and those with propofol (n = 15). From patients classified as physical status I, peripheral blood samples were drawn before anesthesia and at the first postoperative day. Plasma IL-6 concentration was analyzed using the CBA kit, by flow cytometry. DNA was isolated by genomicPrep Min Spin Blood kit for evaluation *of IL-6 -174 G/C* and *IL-6R D358A* polymorphisms by PCR-RFLP and *IL-6 -572 G/C* by Taqman real time PCR. Statistically significant increase of IL-6 concentration was observed at the following day of surgery in both groups. No association was detected between plasma IL-6 and the three polymorphisms. In conclusion, anesthesia with sevoflurane or propofol released plasma IL-6, whereas IL-6 and IL-6R polymorphisms seemed not to be associated with the inflammatory status.FAPESP.

P94

Effect of Cisplatin and Gemcitabine on DNA Structure and hMLH1 and RASSF1A Gene Expression in Urinary Bladder Carcinoma Cell Lines. da Silva GN, de Camargo EA, Gobette CP, Marcondes JPC, Salvadori DMF. Department of Pathology, FMB, UNESP, Botucatu, SP, Brazil.

Currently, a combination of cisplatin and gemcitabine is considered the standard protocol for treating bladder cancer. The aim of this study was to investigate the genotoxicity of these two antineoplastic drugs, as well as to study their effects on the expression and methylation patterns of the RASSF1A and hMLH1 genes. Three different bladder carcinoma cell lines carrying a wild-type TP53 (RT4, grade 1) or mutant TP53 alleles (5637, grade 2; T24, grade 3) were used. The comet assay, MSP-PCR and qPCR-RT were used to analyze the effects of cisplatin (1.0 μM), gemcitabine (1.56 μM) alone or simultaneously. Data showed that gemcitabine was genotoxic in the three cell lines, regardless of the mutation status of the TP53 gene; cisplatin was genotoxic in RT4 cells and in 5637 cells. The combined treatment was genotoxic in RT4 cells but not in 5637 cells, and decreased DNA damage in T24 cells when compared to control. Treatment with cisplatin or a combination of cisplatin and gemcitabine resulted in decreased hMLH1 mRNA levels in the wild-type-TP53 cells. The changes in hMLH1 gene expression were not related to promoter methylation. Promoter hypermethylation resulting in a lack of RASSF1A gene expression was, however, found in all three cell lines. In conclusion, cisplatin and gemcitabine genotoxicity was independent of TP53 status. Moreover, cisplatin alone and the combined treatment inhibited the expression of hMLH1 in grade 1 cells. However, the absence of RASSF1A gene expression occurred independently of the tumor grade. The antineoplastic protocols used in this study did not change the epigenetic and genetic patterns of the genes investigated. FAPESP/CNPq.

P95

Expression of XRCC5 in Peripheral Blood Lymphocytes Is Upregulated in Subjects from a Heavily Polluted Region in the Czech Republic. Rossner, Jr. P, Uhlirova K, Beskid O, Rossnerova A, Svecova V, Sram RJ. Institute of Experimental Medicine, Prague, Czech Republic.

We investigated the levels of oxidative stress markers [8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG), 15-F2t-isoprostane (15-F2t-IsoP), protein carbonyls] and cytogenetic parameters [genomic frequency of translocations (FG/100), percentage of aberrant cells (%AB.C.) and acentric fragments (ace)] in subjects living in Prague (64 subjects) and in the heavily polluted Ostrava region (75 subjects). We compared the expression of genes participating in base excision repair and non-homologous end-joining. We measured oxidative stress markers by ELISA, cytogenetic parameters by fluorescence *in situ* hybridization and gene expression by quantitative PCR. The levels of air pollutants (benzo[a]pyrene, B[a]P; carcinogenic polycyclic aromatic hydrocarbons, c-PAHs; benzene) measured by personal monitors were significantly elevated in Ostrava compared to Prague ($p<0.001$). Despite this fact, we observed no differences in biomarkers of oxidative stress between the two locations. Moreover, subjects from Ostrava were less likely to have above-median levels of %AB.C. (OR; 95% CI: 0.18; 0.05-0.67; $p=0.010$). Multivariate analyses revealed that subjects living in Ostrava had increased odds of having above-median levels of XRCC5 expression (OR; 95% CI: 3.33; 1.03-10.8; $q=0.046$). Above-median levels of 8-oxodG were associated with decreased levels of vitamins C (OR; 95% CI: 0.37; 0.16-0.83; $p=0.016$) and E (OR; 95% CI: 0.25; 0.08-0.75; $p=0.013$), which were elevated in subjects from Ostrava. We suggest that air pollution by c-PAHs affects XRCC5 gene expression, which probably protects subjects from Ostrava against the induction of a higher frequency of translocations; elevated vitamin C and E levels in the Ostrava subjects decrease the levels of 8-oxodG. Supported by the Czech Ministry of Education 2B08005.

P96

Genotoxic Evaluation of Occupational Exposure to Antineoplastic Agents and Its Association with DNA Repair Genes Polymorphisms. Aristizabal AF, Rangel NE. University of Environmental and Applied Sciences, Bogota, DC, Colombia.

The aim of this study was to evaluate DNA damage in a population of individuals employed in oncology units in the city of Bogotá, Colombia and compare it with a control group. Due to genetic variants can determine the response to DNA damage we studied the possible effect of polymorphisms of repair genes XRCC1 and XRCC3 on damage levels of genetic material. Some characteristics and confounding factors such as age, gender, alcohol consumption, smoking, and exercise routines were also taken into account. Peripheral blood samples were obtained from 40 people between exposed workers and people from the control group. The comet essay was performed using isolated lymphocytes, for which the samples were embedded in agarose and placed on a glass slide; then they were exposed to lysis with detergent solution and finally subjected to an electric current with alkaline buffer. The genotyping for XRCC1 and XRCC3 genes was performed by PCR-RFLP. Compared with those of the control group, partial results of exposed personnel evaluations show a significant increase in DNA damage. It was not observed any influence of age, gender or time exposure on the results of the comet essay. The presence of the XRCC1 polymorphism was associated with the increase of genotoxic effects of these substances, generating a greater individual susceptibility to the undesirable effect of dangerous agents. The results suggest that occupational exposure to antineoplastic drugs without the appropriate security measures can be a high risk to human health.

P97

Gamma-Hexachlorocyclohexane Promotes Downregulation of p53 and Nuclear Translocation of BAX in LnCap Cells. Joseph S[1], d'Auvergne O[2]. [1]Department of Environmental Toxicology, Southern University and A&M College, Baton Rouge, LA, United States, [2]Department of Biological Sciences, Southern University and A&M College, Baton Rouge, LA, United States.

A positive correlation of organochlorine exposure and carcinogenicity and progression is currently ambiguous leading many of such chemicals to be listed only as probable carcinogens due to their bioaccumulative properties. Gamma-hexachlorocyclohexane is currently utilized as a pharmaceutical agent that treats parasitic infestations such as scabies and lice. Previous research shows that moderate to high doses of lindane provoke aberrant changes within the micronuclei of breast and prostate cells without causing cytoxicity. With this investigation, we seek to study the effects of gamma-HHC on BAX and p53 expression as well as the ability of the chemical to promote aggressive behavior in hormone related cancers. To support the goals of this research endeavor, GT1-7 hypothalamic neuronal and LnCap prostate cancer cells were treated with 2.5 and 5 microliters per milliliter of a .2mM concentration of gamma-HHC. At the end of 24 hour incubation, cellular secretions were collected from the GT1-7 populations and induced into populations of LnCap cells. GT1-7 cells were transformed with a pGEM-luc plasmid and promoter activity was studied using a luciferase assay. Immunohistochemical staining was executed to investigate the effects of the chemical on the BAX apoptotic pathway. Rt-pcr was performed to study p53 expression in the LnCap cells after exposure to GT1-7 cellular secretions. p53 was downregulated in a dose dependent manner in LnCap cells. BAX remained within the cytosol of LnCap cells treated with gamma-HHC alone. However, BAX proteins showed a significant amount of nuclear translocation in LnCap cells exposed to cellular secretions of treated GT1-7 cells.

P98

Phenotypic Characterization of Human Polymorphic P450 Genes Using Budding Yeast. Fasullo M[1], Smith A[1], Egner P[2], Cera C[1]. [1]State University of New York, Albany, NY, United States, [2]Johns Hopkins University, Baltimore, MD, United States.

Cytochrome P450 genes are highly polymorphic and specific polymorphisms are correlated with higher cancer incidence. Whether specific polymorphisms enhance the metabolic activation of carcinogens is often difficult to discern from epidemiological data. To determine whether specific P450 polymorphisms affect the genotoxicity of environmental carcinogens, we individually expressed CYP1A2, CYP1A1 and CYP3A4 in budding yeast (*Saccharomyces cerevisiae*), which do not contain P450 genes. Methods: We measured genotoxic endpoints, including carcinogen DNA adducts, Rad51 foci, DNA damage-associated recombination and mutation, after acute exposure to environmental carcinogens, aflatoxin B[1] (AFB[1]) and benzo[a]pyrene dihydrodiol (BaP-DHD). We measured growth and survival of yeast DNA repair mutants expressing P450 genes after chronic exposure of low concentrations of carcinogens. We studied both CYP1A2 polymorphisms due to amino acid changes and CYP1A1 polymorphisms associated with breast cancer. Results: After yeast expressing CYP1A2 polymorphisms were exposed to carcinogens, our data indicated a strong correlation between DNA adduct levels, carcinogen-associated recombination, and decreased survival. We detected carcinogen-associated Rad51 foci in P450-expressing cells after two hours of exposure. After chronic exposure to low concentrations of carcinogens, we observed differences in survival of yeast mutants, deficient in both recombination and nucleotide excision repair, expressing particular P450 alleles. Discussion: We deduced that differences in P450-mediated carcinogen genotoxicity can be measured in yeast expressing specific P450 alleles, and CYP3A4 expression is sufficient to metabolically activate carcinogens in yeast. These studies thus enable a new approach for studying P450 polymorphisms.

P99

Association of Cytochrome P4501A1 Exon 7 Gene Polymorphisms with Non-Small Cell Lung Cancer. Mundluru HP[1], Peddireddy V[1], Somanath N[2], Badabagni SP[1], Kadali KD[1], Gundimeda SD[2], Varre SD[1], Penagaluru PR[3], Akka J[1]. [1]Institute of Genetics, Hyderabad, Andhra Pradesh, India, [2]Indo-American Cancer Hospital, Hyderabad, Andhra Pradesh, India, [3]Bagwan Mahavir Hospital and Research Centre, Hyderabad, Andhra Pradesh, India.

Susceptibility to lung cancer has been influenced by inheritance of polymorphic genes encoding cytochrome P450 1A1 (CYP1A1) which is involved in the bioactivation of environmental toxins and carcinogens. Cytochrome P450 (CYP) enzymes are responsible for the metabolic activation of numerous xenobiotics, endogenous compounds and major classes of tobacco carcinogens. Several polymorphisms in the CYP1A1 locus have been identified and their genotypes exhibit population frequencies that depend on ethnicity. In the present investigation 246 lung cancer patients and 250 age and sex matched healthy controls were studied for CYP1A1 polymorphisms in the population of Andhra Pradesh. Information on their age, type of cancer and habits like smoking and alcohol were recorded using a standard questionnaire. DNA extraction was carried out and gene polymorphisms of CYP1A1, A→G transition in exon 7 were determined by PCR-RFLP according to the method of Nansong *et al.* (2001). The frequencies of AA and GG genotypes (homozygous) were 29.7% and 12.2% in lung cancer patients in contrast to 78.4% and 8% in control subjects, respectively. The frequency of AG genotype (heterozygous) was 58.4% in lung cancer patients and 13.6% in controls. A considerable difference in the genotypic distribution of AG genotypes was observed in patients (OR=8.82, 95% CI: 5.67-13.72, p=0.001). CYP1A1 m2 gene polymorphisms demonstrated a significant association with the heterozygous 'AG' genotype in the lung cancer patients showing 8.8 fold risk of disease susceptibility. The CYP1A1 m2 heterozygous condition may be an important risk factor for lung cancer.

P100

SNP Associations with Aristolochic Acid Nephropathy. Rosenquist TA[1], Jelakovic B[2], Grollman AP[1]. [1]Stony Brook University, Stony Brook, NY, United States, [2]University of Zagreb, Zagreb, Croatia.

Introduction. Aristolochic acid (AA), a nitrophenanthrene carboxylic acid of *Aristolochia* species, is the environmental toxin responsible for the syndrome known as endemic (Balkan) nephropathy (EN). And ingestion of AA in herbal remedies leads to aristolochic acid nephropathy (AAN) a global health problem. Both disorders are associated with upper urinary tract urothelial carcinomas (UUC). A genetic component to EN and AAN has been recognized and we will present evidence implicating two genomic regions containing genes contributing to AA-susceptibility. Methods. DNA samples from a selection of 144 EN cases and 449 control subjects from the endemic region of Croatia were collected. The genotypes in these DNAs of a set of 1526 single nucleotide polymorphisms (SNPs) were determined. The SNPs were chosen from regions throughout the genome containing candidate genes for involvement in AA-metabolism or AAN disease progression as well as regions orthologous to mouse QTL important in the mouse model of AAN. Results. Two regions were identified as containing SNPs associated with occurrence of AAN. One region contains the PLA2A gene cluster on Chr 1. The other is in the EPB41L4B gene on Chr 9, a region corresponding to a mouse AAN QTL. Discussion. AA has long been known used as a competitive inhibitor of PLA2 activity. The relevance of this to nephropathy is not known. These results implicate PLA2 genes in the genesis of AAN. The finding of a human association in a region orthologous to a mouse QTL for AAN validates the use of mouse genetics to study AAN.

P101

Evaluation of MMP-7 Gene Promoter Polymorphism in Chronic Pancreatitis. Venkateshwari A[1], Sri Manjari K[1], Pratibha N[2], Vidya Sagar A[3], Jyothy A[1]. [1]Institute of Genetics & Hospital for Genetic Diseases, Osmania University, Hyderabad, Andhra Pradesh, India, [2]Department of Genetics, Osmania University, Hyderabad, Andhra Pradesh, India, [3]Department of Gastroenterology, Gandhi Hospital, Hyderabad, Andhra Pradesh, India.

Introduction: Chronic Pancreatitis is a progressive disease characterized by irreversible destruction of exocrine pancreatic tissue and extensive fibrosis. MMP-7 is a protease with broad substrate specificity, being able to degrade elastin, proteoglycans, fibronectin, and type IV collagen. The functional single nucleotide polymorphism (SNP) has been identified in the promoter of MMP-7, rs11568818 (-181 A/G) which has been shown to modulate transcription by influencing the binding of nuclear proteins. Thus the aim of the present study is to investigate possible genetic association of matrix metelloproteinase-7(MMP7) gene promoter polymorphism in chronic pancreatitis. Methods: The study included 112 chronic pancreatitis patients and an equal number of healthy control subjects. The SNP in the MMP-7 promoter was analyzed using PCR-RFLP. The genotype and allele frequencies were compared between cases and controls using appropriate statistical analysis. Results: The frequencies of polymorphic genotypes in promoter of MMP 7 were AA=55.4%, AG =25 % and GG = 19.6 % in chronic pancreatitis patients and AA= 69.6 %, AG =25 % and GG =5.4 % in control subjects. There is an increased frequency of G [OD = 0.46 (0.288 - 0.731), p = 0.001*] allele in the disease compared to control subjects, indicating its possible association with the disease. Discussion: These findings suggest that MMP-7 gene promoter polymorphism may play an important role in the occurrence of the disease *via* increased matrix degradation and tissue damage. Thus, G allele of MMP 7 may act as a susceptibility factor in the etiopathogenesis of chronic pancreatitis.

P102

Genetic Polymorphisms of Metabolic Enzymes GSTT1, GSTM1, CYP2E1, and MTHFR in a Group of Children with Acute Lymphoblastic Leukemia and Its Relationship with the Disease. Sánchez MP[1], Groot de Restrepo H[1], Uribe GI[2]. [1]Universidad de los Andes, Bogotá, Colombia, [2]Hospital de la Misericordia, Bogotá, Colombia.

Acute lymphoblastic leukemia (ALL) accounts for 25% of childhood cancer. Methylenetetrahydrofolate reductase (MTHFR) has a major impact on the regulation of the folic acid pathway due to conversion of 5,10-methylenetetrahydrofolate to 5-methyl-THF. Two polymorphisms (677C>T and 1298A>C) in the gene coding for MTHFR have been shown to reduce MTHFR activity and were associated with the susceptibility to ALL. We studied the influence of genetic polymorphisms in the *MTHFR* and the genes *GSTM1, GSTT1, as* well as the *CYP2E1,* on the frequencies of children with ALL. We performed a case-control study including 147 patients with ALL and a control group of 160 individuals. The ALL samples were obtained from *La Misericordia* hospital in Bogotá - Colombia. DNA from peripheral blood leucocytes was used in PCR followed by *HinfI* and *MboII* restriction digestion to determine the MTHFR 677 and 1298 genotypes respectively. Odds ratios (OR) and 95% confidence intervals were calculated. Statistics analysis to determine whether there was an association between the MTHFR, GSTT1, GSTM1 and CYP2E1 polymorphisms, as well as environmental factors such as exposure to chemical substances and smoke, proximity to factories, alcohol consumption, with the recurrence of ALL. We found no association between the enzyme polymorphism GSTT1 [OR= 0.91; (0.50-1.66)], CYP2E1 [OR= 1.43; 0.88-2.32)] or MTHFR C677T [OR= 0.69; (0.44-1.10)] - A1298C [OR= 1.13, (0.60-2.13)] with ALL. However, we observed a significant association between the GSTM1 polymorphism [OR= 2.39; (1.44-3.96)] with exposure to some environmental factors (cigarette smoke and proximity to factories) and the appearance of ALL in children.

P103

Accumulation of the MtDNA 4977 Bp Deletion-Induced by Ionizing Radiation in Human Blood. Wang P[1], Jiang F[2], Han L[1], Wang XA[1], Yang ZZ[1], Lu YM[1]. [1]Henan Institute of Occupational Medical, Zhengzhou, Henan Province, China, [2]College of Public Health, Zhengzhou University, Zhengzhou, Henan Province, China.

Introduction: To investigate the change of the mtDNA 4977bp deletion in human peripheral blood cells exposed to ionizing irradiation, and explore the feasibility of 4977bp deletion changes as a biodosimeter. Methods: Human peripheral whole blood samples were collected from six healthy individuals, and exposed to ^{60}Co γ-rays with dose from 0 to 5Gy. The genetic DNAs were isolated from the samples and the number of mtDNA 4977bp deletion and total mtDNA number were detected by real-time PCR at 2h after irradiation. Results: The number of mtDNA 4977bp deletion in human peripheral blood cells increased gradually with the dose from 0 to 3Gy after irradiation ($F=12.796$, $P<0.01$). Meanwhile, it accumulated with time from 2h to 72h ($F=11.753$, $P<0.01$). However, the deletion rates at 2h were not significantly different between different dose groups. In addition, the rate of mtDNA 4977bp deletion in peripheral blood cells from 87 healthy donors was lower than 3.076×10^{-5}%. Discussion: The change of mtDNA 4977bp deletion in human peripheral blood cells could be induced by ionizing radiation; it was accumulated with dose from 0 to 3Gy. So it might be a potential biodosimetry candidate applicable in early-response radiation accident victims.

P104

Relationship between Gene Polymorphisms and DNA Damage in Morbid Obese Women. Almeida DC, Luperini BCO, Salvadori DMF. São paulo State University, Botucatu, SP, Brazil.

It is accepted that genetic and environmental factors predispose individuals to the development of obesity. It is known that many genes are involved in predispositions, and some (such as ghrelin, ghrelin receptor, leptin and FTO) play important roles. Ghrelin, a gastrointestinal hormone, is involved in food intake and energy balance regulation; leptin, another hormone, acts in the anorexigenic pathway by decreasing appetite and increasing energy expenditure; FTO polymorphisms have been associated with Body Mass Index (BMI) and obesity. Using the comet assay, this study aimed to evaluate the DNA damage in 70 morbid obese women (BMI ≥ 30 kg/m^2) and in 70 women with normal BMI (BMI < 25 kg/m^2; control group) and to correlate these lesions with the ghrelin, ghrelin receptor, leptin and FTO gene polymorphisms, according to the genotypes. Our findings showed statistically significant higher amount of DNA damage in morbid obese women (28.92 ± 33.09) when compared to control group (16.94 ± 23.51). When ghrelin gene polymorphism was assessed, we observed that individuals with CC genotype presented higher amount of damage (32.79 ± 34.14) than those AC (28.07 ± 33.20) and AA (28.31 ± 32.51); for ghrelin receptor, leptin and FTO gene polymorphisms, those subjects with the genotype AC (30.70 ± 33.39), GG (37.22 ± 35.77) and AA (36.70 ± 34.12), respectively, had higher levels of DNA lesions than the other genotypes. Taken together, the results suggest association between genotypes and increased incidence of genotoxic damage in obese women.

P105

DNA Damage and Its Relationship with Morbid Obesity. Luperini BCO, Almeida DC, Salvadori DMF. São Paulo State University, Botucatu, SP, Brazil.

Obesity is a major health problem that increases the risk of several common diseases such as type 2 diabetes, heart diseases, hypertension, metabolic syndrome, and cancer. Over the last years, the prevalence of overweight and obesity have increased rapidly. The rise in human obesity is caused by decreased energy expenditure and increased energy intake that result in an increase of adipose tissue that is harmful to human health. Obesity is commonly assessed by calculating individual Body Mass Index (BMI) (kg/m ^{2}). Individuals with a BMI ≥ 25 kg/m^2 are classified as overweight, and those with a BMI ≥ 30 kg/m2 are considered obese. Some studies have reported that obesity can lead to the production of free radicals, which could interact with DNA causing genotoxic damage. We studied DNA damage in peripheral lymphocytes of 70 morbid obese women and 70 controls, using the comet assay improved with lesion-specific endonucleases (formamidopyrimidine DNA-glycosylase (FPG) and endonuclease III (ENDOIII). Results showed significant higher amount of oxidized purines and pyrimidines in obese patients than in the control group (P < 0.01). In conclusion, our data demonstrated an association between morbid obesity and increased oxidative DNA damage in peripheral lymphocytes, suggesting higher susceptibility of obese patients to develop genetic-related diseases than subjects with BMI lower than 25 kg/m^2.

P106

Copy Number Changes across the Mouse Genome Discovered Using the Mouse Diversity Genotyping Array Show Tissue and Genotype Specificity. Wishart AE, Eitutis SE, Hill KA. The University of Western Ontario, London, ON, Canada.

To date, *in vivo* mutation detection has used single-gene mutation targets and primarily neutral transgenes as sentinels for studying the frequency and pattern of mutations across the genome. These single-gene approaches are too narrow a perspective to detect large genomic changes, including germline copy number variants (CNVs) and somatic copy number changes (CNCs). We applied the novel Mouse Diversity Genotyping Array (MDGA), containing over 900,000 unique probes to detect copy number, as a mutation detection system to compare CNCs across the genome between two somatic tissues of the same mouse. The burden and nature of CNCs were examined in two male harlequin (hq) mice deficient in Apoptosis-inducing factor resulting in mitochondrial dysfunction, neurodegeneration and premature aging, and compared with two AIF-proficient littermates. Genomic segmentation analysis using Partek® Genomics Suite detected similar numbers of CNCs between spleen and cerebellum in both hq and AIF-proficient mice. The profile of CNCs was more similar between the two hq than between the two AIF-proficient mice. The number of gains in copy number was similar to losses in AIF-proficient mice, but gains exceeded losses in hq mice ($p<0.001$), specifically in the cerebellum of hq mice compared to that of AIF-proficient mice ($p<0.001$). There also were more deletions in copy number in the cerebellum of hq cerebellum compared to AIF-proficient mice ($p<0.001$). A hq cerebellum-specific CNC profile was detected, potentially relevant to the neurodegenerative phenotype. The MDGA approach to mutation detection identifies a greater spectrum of mutation types mostly absent in the majority of traditional approaches.

P107

Hydroxyurea Induces *De Novo* Copy Number Variants in Human Cells. Arlt MF, Ozdemir AC, Birkeland SR, Wilson TE, Glover TW. University of Michigan, Ann Arbor, MI, United States.

Copy number variants (CNVs) are distributed throughout the genome, where they contribute to genetic variation and phenotypic diversity. Spontaneous CNVs are a major cause of genetic and developmental disorders and birth defects, and arise frequently in cancer cells. Like all mutation classes, environmental and genetic factors almost certainly increase the risk for new, deleterious CNVs. Despite their importance, there is limited understanding of these risk factors and the mechanisms responsible for most CNVs. Here we report that low doses of hydroxyurea (HU), a ribonucleotide reductase inhibitor and an important drug in the treatment of sickle cell and other diseases induces a high frequency of *de novo* CNVs in cultured human cells that resemble pathogenic CNVs in size and breakpoint structure. We exposed normal fibroblasts to HU-mediated replication stress and clonal cell populations were analyzed for CNVs using SNP arrays. We found that HU concentrations equivalent to those in patient serum, resulted in 1.8-2.4 *de novo* CNVs/clone, a significant increase over the 0.7 CNVs/clone seen in controls ($p<0.001$). These CNVs are distributed throughout the genome, with some hotspots. Breakpoint junctions showed microhomologies, blunt ends, and short insertions, consistent with a major class of human CNVs that are generated by nonhomologous repair mechanisms. Together with our published findings with aphidicolin, these data suggest that any environmental agent causing replication stress has the potential to induce deleterious CNVs. In addition, they point to a need for further study of the potential risk for *de novo* CNVs in HU-treated patients and their future generations.

P108

Functional Genomics Approach to Elucidating Mechanisms of Trichloroethylene Toxicity. De La Rosa VY, Vulpe CD. UC Berkeley, Berkeley, CA, United States.

Trichloroethylene (TCE) is an industrial solvent and a common drinking water contaminate. Previous studies identified the TCE metabolites DCVG and DCVC as the moieties causing increased kidney toxicity and cancer, yet the underlying molecular events remain unknown or debated. Using a functional genomics approach in yeast, parallel deletion analysis, we aim to obtain a better understanding of the mechanisms involved in DCVG and DCVC toxicity. As many of the pathways and gene functions in yeast are conserved in higher eukaryotes, results from this analysis can inform studies in higher organisms. The yeast deletion library was treated with an acute exposure of DCVG or DCVC for 5 generations followed by computational analysis. Enrichment analysis of both genetic profiles revealed genes involved in nucleotide excision repair, post-replication DNA repair and DNA damage response. Individual growth confirmations of these genes such as *Rad18, Rad5, Rev1,* and *Rad10* showed increased sensitivity to both metabolites, implicating DNA damage as a possible mechanism of toxicity. In addition, similar gene sensitivities were seen in experiments with formaldehyde and other DNA damaging agents. *Rad18, Rad5* and *Rev1* function as critical components of error prone and error free post-replication repair pathways in yeast and humans, suggesting DCVG and DCVC may cause DNA lesions that lead to genome instability and toxicity. Future work will focus on identifying the favored mechanism of repair and the type of lesions caused by these metabolites. Altogether this work will provide insight on potential genotoxic mechanisms leading to TCE-induced kidney toxicity.

P109

Evolution of Colon Cancer Is Reflected in DNA Mutational Sequence Profiles. Elespuru RK[1], Rajani A[1,2]. [1]U.S. Food and Drug Administration, Silver Spring, MD, United States, [2]University of Maryland Baltimore County, Baltimore, MD, United States.

Fearon and Vogelstein proposed an evolutionary scheme for colorectal cancer 20 years ago. This included different stages of pathological change, accompanied by genetic changes in oncogenes or tumor suppressor genes. This scheme has been corroborated and expanded (*e.g.* Markowitz and Bertagnoli, *NEJM* 2009). It is notable that the first set of mutations in the pathway involves mismatch repair genes, the same deficiencies occurring in families with elevated colon cancer susceptibility. The hypothesis proposed is that the sequence changes occurring in later stages will be altered in specific ways reflecting the absence of mismatch repair mechanisms. In particular, G-T mismatches would not be repaired and DNA sequence changes would be biased toward G to A transitions. Since these mutations are found disproportionately at CpG sites, this bias would be expected to be greater at CpG sites. We have tested this hypothesis by summarizing mutation profiles found in colon cancer from the literature and in databases (IARC, COSMIC), and compared them with mutant profiles found in other cancers (not associated with mismatch repair). Genes examined in the colon cancer pathway, including K-RAS and TP53, contain high levels of G to A transition mutations. In TP53, nearly 60% of all mutations in colon cancer are G to A transitions at CpG sites, independent of geographical origin. Sjoblom *et al.*, 2011, in a genome wide search for tumor-related mutations, found G to A transitions at ~60% in 519 colon cancer genes and ~35% in 673 breast cancer genes. The data support the hypothesis.

P110

Genomic Changes in Primary Human Uroepithelial Cells following 24-Hour Exposure to Mixtures of Arsenite and Its Principal Methylated Metabolites. Yager JW[1], Efremenko A[2], Black M[2], Thomas RS[2], McKim J[3], Wilga PC[3], Arnold LL[4], Gentry PR[5], Clewell HJ[2]. [1]University of New Mexico, Albuquerque, NM, United States, [2]The Hamner Institutes for Health Sciences, Research Triangle Park, NM, United States, [3]CeeTox, Inc., Kalamazoo, MI, United States, [4]University of Nebraska Medical Center, Omaha, NE, United States, [5]Environ International, Monroe, LA, United States.

Objective: To evaluate gene expression changes in primary human uroepithelial cells exposed to arsenite and its methylated metabolites in order to identify cell signal pathway perturbations in common that are potentially associated with bladder carcinogenicity. Methods: Human uroepithelial cells from kidney-donor ureter segments were treated in culture with mixtures of inorganic arsenic and its metabolites for 24 hours at relative proportions (1:1:4) typically observed in the urine of individuals exposed to arsenic in drinking water. Total arsenic concentrations ranged from 0.06 to 18 micromolar. Two series of *in vitro* exposures were conducted: one (5 subjects) with arsenite and the pentavalent methylated metabolites and a second (10 subjects) with arsenite and the trivalent methylated metabolites. Gene expression was determined using Affymetrix human genome microarrays. Results: Similar responses were obtained for mixtures containing pentavalent or trivalent metabolites. Principal component analysis indicated that the variation across individuals was substantially greater than the changes in expression elicited by arsenic treatment. However, a suite of gene changes was identified that reflects the effects of sub-micromolar arsenic on a number of key signaling pathways across nearly all subjects: HMOX1 (oxidative stress), FKBP5 (protein folding), LGALS8 (growth regulation), MT1E (metallothionine regulation), DDB2 (DNA damage sensing), TXN/TXNRD1 (thioredoxin regulation) and THBD (immune response). The concentration/response for DDB2 and THBD were non-monotonic, with reversal in direction of effect around 0.1 micromolar. Conclusion: Together, these genes may serve as an early biomarker of effects for arsenic. Work is underway to investigate changes in gene expression over time.

P111

A Novel Approach to Assess NQO1 Responses to Arsenic Utilizing a Human Uroepithelial Primary Cell Culture System. Balbuena P[1], Clewell R[1], Efremenko A[1], Pluta L[1], Black M[1], Gentry PR[2], Clewell HJ[1], Yager JW[3]. [1]The Hamner Institutes for Health Sciences, Research Triangle Park, NC, United States, [2]Environ International, Monroe, LA, United States, [3]University of New Mexico, Albuquerque, NM, United States.

Inorganic arsenic in drinking water has been associated with increased incidence of bladder cancer in humans originating in uroepithelial cells. We have developed a cell culture method that allows primary human uroepithelial cells (HUECs) from individual subjects to be passaged several times, increasing the number of cells available for evaluation of gene and protein response. Cells from three human donors were passaged up to three times and grown to confluency. Gene profiling was performed on each passage without any treatment to assess gene expression changes due to passaging of cells. Cells did not show differences in gene expression in response to passaging. Passaged cells were treated in culture for 24 hours with inorganic arsenic and its metabolites individually and in a mixture with relative proportions (1:1:4) as observed in the urine of exposed individuals and assessed for changes in NQO1 protein expression. NQO1 (NAD(P)H dehydrogenase [quinone] 1) prevents the formation of radical species and altered expression of this protein has been seen in many tumors. Expression of NQO1in response to treatments with arsenite and its metabolites increased for all treatments in a concentration-responsive manner. This novel system should prove valuable in the assessment of different cellular endpoints in primary HUECs. Its use greatly reduces potential variability inherent in the use of samples from different individuals, and eliminates the need for large numbers of tissue samples.

P112

Genotoxicity and Mutagenicity of Three Nitrosamines and Their Analogous Nitramines. Osiol JL[1], Mitch WA[2], Wagner ED[1], Plewa MJ[1]. [1]University of Illinois, Urbana, IL, United States, [2]Yale University, New Haven, CT, United States.

Nitrosamines are an important class of emerging environmental contaminants. Many are recognized as carcinogens and found in a variety of places - food, cigarette smoke, and drinking water, to name a few – that are relevant to human exposure. A less-studied but related class of compounds, the nitramines, is structurally similar to the nitrosamines. These agents are generally considered to be less important toxicologically than the nitrosamines but are much more stable and therefore more persistent than their related nitrosamines. In this study, three nitrosamines (dimethylnitrosamine, nitrosomorpholine, and 1,4-dinitrosopiperazine) and their nitramine analogs (dimethylnitramine, nitromorpholine, and 1,4-dinitropiperazine) were assayed for mutagenicity and genotoxicity. Mutagenicity was measured by a microplate suspension test using *Salmonella typhimurium* strain YG7108, a strain developed specifically for sensitivity to the nitrosamines, and a mammalian metabolic activation system (S9 microsomes from rats induced with Aroclor). Genotoxicity was conducted in Chinese hamster ovary (CHO) cells utilizing an optimized mammalian metabolic activation system (S9B150) and measured with single cell gel electrophoresis (SCGE). The three nitrosamines were mutagenic in *Salmonella* and genotoxic in CHO. The nitramines were mutagenic in *Salmonella*, though more weakly than their nitrosamine partners. When assayed in the CHO cells, the nitrosamines were positive but the nitramines were negative. These results indicate that the nitramines can behave like the nitrosamines and produce a mutagenic response in *S. typhimurium*, yet not genotoxic in the mammalian cell line. Nitrosamines remain the more potent agents of concern, yet there is a potential genotoxic hazard associated with the nitramines as well.

P113

Genotoxic Mechanism of Haloacetic Acid Drinking Water Disinfection Byproducts. Dad A, Pals J, Wagner E, Plewa MJ. University of Illinois, Urbana, IL, United States.

Recently we reported the biological mechanism for the genotoxicity of haloacetic acid (HAA) drinking water disinfection by products as the inhibition of glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Inhibition of GAPDH by bromoacetic acid (BAA) blocks glycolysis and the cell cannot produce pyruvate. Pyruvate is essential for aerobic respiration. The inhibition of GAPDH generates reactive oxygen species (ROS) and subsequently damage to DNA. To further test this hypothesis we treated Chinese hamster ovary (CHO) cells with BAA concentrations (60 – 70 μM) that inhibited GAPDH and induced genomic DNA damage as measured by the Comet assay. Cells were also treated with pyruvate alone (10-20 mM), and with pyruvate plus BAA. Results showed that pyruvate was neither genotoxic nor cytotoxic. The cells treated with the combination of BAA and pyruvate had less DNA damage than the cells treated with only BAA. We concluded that BAA inhibited GAPDH and cells with depleted pyruvate expressed an enhanced level of DNA damage. Cells exposed to exogenous pyruvate eliminated the genotoxic impact under conditions of GAPDH inhibition. The results of this pyruvate rescue experiment support the hypothesis that GAPDH is the cellular target of BAA-induced genotoxicity.

P114

Cytotoxicity and Genotoxicity of Disinfection By-Products in Drinking Water in Europe: HIWATE. Jeong CH[1], Anduri S[2], Richardson SD[2], Nieuwenhuijsen MJ[3], Kogevinas M[3], Wagner ED[1], Plewa MJ[1]. [1]University of Illinois, Urbana, IL, United States, Urbana, IL, United States, [2]U.S. Environmental Protection Agency, Athens, GA, United States, [3]Centre for Research in Environmental Epidemiology, Barcelona, Spain.

HiWATE (Health impacts of long-term exposure to disinfection by-products in drinking Water) project is the first systematic analysis that combines the epidemiology on adverse pregnancy outcomes with analytical chemistry and toxicology in the European Union. To investigate the hypothesis that drinking water associated with adverse human reproductive and developmental outcomes also have enhanced metrics of *in vitro* toxicity, total 11 water samples were collected from various countries where different disinfection methods are applied. Each location corresponds to the region which cohorts of epidemiological study on adverse reproductive effects are conducted. Extraction and chemical analysis were performed and broad ranges of DBPs (Disinfection By-Products) were identified. The results showed that a diversity of DBPs is generated in different source waters that are treated with different disinfectants. Sample 5 corresponded to a control cohort contained the lowest number of DBPs. To investigate the possible correlation between the analytical chemistry and toxicology, we compared the *in vitro* chronic cytotoxicity and acute genomic DNA damages in mammalian cells with the HiWATE samples. The results demonstrated a significant correlation between the cytotoxicity and genotoxicity. There was a good correlation of cytotoxicity and numbers of DBPs, and a moderate correlation of genotoxicity and numbers of DBPS. Future study will investigate the possible association between chronic cytotoxicity, acute genotoxicity, and epidemiology in terms of specific chemicals by sorting these data by chemical classes.

P115

A Population-Level Genetic Model of Low Dose Co-Exposures Reveals a Genetic Basis for Increased Cancer Susceptibility. DeSimone MC, Mashburn Z, Wadsworth K, Patisaul H, Threadgill DW. North Carolina State University, Raleigh, NC, United States.

Traditional experimental models that examine low dose co-exposures to environmental carcinogens have lacked the genetic component critical for the discovery and identification of genes underlying disease susceptibility. To model inter-individual variation in metabolism, detoxification, and transport, as well as genetic heterogeneity of exposed human populations, we developed a unique intercross population derived from FVB/N$^{Abcb1a/1b-/-}$, a multi-drug resistant p-glycoprotein knockout mouse model, and CAST/EiJ a wild-derived strain that is genetically distinct from the FVB/N background. By modulating drug transport and genetic variation in the mouse to model human biology, including using a western diet, environmentally-relevant doses of carcinogens, trichloroethylene (TCE) and inorganic arsenic, can be evaluated for their effects on toxicity susceptibility. A study cohort of 900 FVB/N$^{Abcb1a/1b-/-}$ x CAST/EiJ F3 mice was divided into nine dose groups, each containing 50 female and 50 males, and was administered TCE and sodium arsenite *via* the drinking water and chow, respectively, at environmentally-relevant concentrations for 56 weeks using a dose-ratio approach. At harvest, blood, feces and all tissues were either formalin fixed or flash frozen for biomarker and histological analysis. We identified a spectrum of treatment-related tumors and lesions in the liver, kidney, lung, breast, testis and skin of populations co-exposed to TCE and arsenic. Mice co-exposed to arsenic in combination with TCE also showed a 2- to 7-fold increase in the urinary biomarker of kidney injury, lipocalin-2, after 16 weeks of treatment. Genetic analysis of individual mice within each population will reveal susceptibility loci responsible for the development of tumorigenesis.

P116

***In Vivo* Antimutagenic Effect of Vitamins B_1, B_6 and B_{12} on Urinary Mutations Induced by Norfloxacin and Nalidixic Acid in CD1 Mice.** Arriaga Alba M, Ruiz Perez NJ, Sanchez Navarrete J. Hospital Juarez de México, México, DF, Mexico.

Vitamins are well known to reduce reactive oxygen species (ROS) exposure. Nevertheless, vitamin B antimutagenesis has not yet been widely explored. The aim of this work was to evaluate the antimutagenicity properties of B_1, B_6 and B_{12} against the quinolone antibiotic Norfloxacin (NOR) and its precursor compound Nalidixic acid (NLX) against urinary mutations in CD1 mice. *In vivo* antimutagenesis studies were performed using *S. typhimurium* TA102 to detect urinary mutagens induced by NOR (70 mg/kg) or NLX (100 mg/kg) after acute daily oral administration for three days in CD1 mice. Cyclophospamide (50mg/Kg), orally administrated, was employed as a positive control. Urine samples were also tested for strain TA102 survival. Vitamins B_1 or B_6 were given orally at doses of 10 or 100 mg/Kg), alone or simultaneously with antibiotics. Vitamin B_{12} was given orally (4 mg/Kg) alone or with the antibiotics. Results showed that vitamins B_1 or B_6 (10 or 100 mg/kg) reduced the levels of urinary mutagens, excreted either free or as β-glucoronidase conjugates, induced by NOR ($P<0.001$) or NLX ($P<0.02$), when administrated with the antibiotics. Vitamin B_{12} reduced urinary mutagen levels induced by NOR ($P<0.02$) or NLX ($P<0.02$). Any of the urine samples were toxic to *Salmonella typhimurium* TA102. The data suggest that vitamins B_1, B_6 and B_{12} inhibited ROS-generated mutations by NLX or NOR, *in vivo*. The employment of mutagenic antibiotics cannot be avoided because of their great therapeutic value, however, simultaneous administration of B vitamins may reduce their genotoxic risks.

P117

How the Plethora of Antimutators in Yeast Led to the Theory of Spontaneous Cancer Remission. von Borstel RC. University of Alberta, Edmonton, AB, Canada.

The Theory of Spontaneous Remission of Cancer arose from the discovery that about 30 percent of genes in yeast can undergo antimutator and mutator activity. The ratio of mutators to antimutators is approximately 5 antimutators for each 3 mutators. Ergo, 60% of the genes affecting mutation rates appear to be antimutators. Ivan Matic has demonstrated that the evolution of bacteria starts with an unsatisfactory stress. As Susan Rosenberg has shown, stress initiates epigenesis to begin the process of turning on mutator genes that eventually turn on more mutators. As Lawrence Loeb has shown, cancer proceeds in the same way, mutators turning on more mutators, with death of many tumour cells as well as the progression of cells to full-blown cancer cells that will enter one or more organs in the patient. Matic has now demonstrated that antimutator genes induced by mutators drive the mutation rate back to an original low spontaneous mutation rate. Out of a million individuals who get cancer, about 100 of them go into total remission. It is possible that the total remission results from the action of antimutator genes. Since the selection of organisms during the evolutionary process is a metaphor for the processes of growth and selection of cancer cells, it seems possible that cancers could be driven into total remission by an accidental or scientifically reliable method for turning on an array of antimutator genes. Thus the spontaneous remissions of cancer, if controlled genetically, should be a key to obtaining spontaneous remission of all cancers.

P118

Genotoxic and Antigenotoxic Effect of the Root of Leatherstem (*Jatropha dioica* Moc. *et Sessé*) Evaluated with the Micronucleus Test. Tenorio-Morales P, Hernández-Romero J, Almaguer-Vargas G, López-Santillán I, Hernández-Ceruelos A. Instituto de Ciencias de la Salud, Universidad Autonoma del Estado de Hidalgo, Pachuca, Hidalgo, Mexico.

Leatherstem, known named in Spanish as Sangre de drago (dragon´s blood), is a small shrub of 1 to 3 ft, with tiny pink or white flowers that appear in clusters during spring and early summer. The root of the plant spreads underground runners to form colonies as wide as 6 ft, and when the sap turns blood red when is exposed to air it turns blood red. The infusions of the root have beenis used to treat alopecia, oral and skin lesions, infections and cancer. The aim of this work was to characterize establish with the micronucleus test the genotoxic and potential chemoprotective capacity of the infusion, using the micronucleus assay with in a subchronic treatment s as it is used in traditional medicine. For the genotoxicity assay, mice were treated as follows: negative control with purified water, positive control, colchicine (10mg/kg), and 3.5, 10.71 and 21.42 ml/kg of root infusion administered by orally route for 45 days. The first week blood samples were collected during the first week of exposure were taken daily and later every week. Results showed a decrease in the EPC/ENC rate byin the 4th day of treatment with the middle and high doses of root infusion, with both indicators returning soon after to recovering the normal levels; thus long term and the genotoxic effects during the rest of the treatment wereas not evident. For the antigenotoxic assay, the same 3 doses of the infusion were tested against plus 30 mg/kg of cycloohfosphfamide were tested. Results showed that the higher dose is able to reduce the induction of EPCMN, nevertheless the EPC/ENC ratio decreased significantly during the first week of treatment. Antigenotoxic effect s after 4 weeks of treatment wereis evident only with the highest dose of root infusion.

P119

Withdrawn.

P120

Mutagenicity of AIDS Therapeutics in a Bacterial Reverse Mutation Assay. Swartz CD[1], Lentz C[1], Baldetti C[1], Garibaldi P[1], Recio L[1], Witt KL[2]. [1]Integrated Laboratory Systems, Research Triangle Park, NC, United States, [2]National Institute of Environmental Health Sciences, Research Triangle Park, NC, United States.

The National Toxicology Program's (NTP) AIDS Therapeutics Toxicity Studies Program involves comprehensive toxicity assessments of therapeutic chemicals used to treat patients with human immunodeficiency virus (HIV) infection. Chemicals are assessed either as single agents or in combinations that are commonly employed in the clinic. As part of this Program, the NTP recently evaluated five chemically diverse AIDS therapeutics - efavirenz (EFV), nelfinavir (NFV), zidovudine (AZT), nevirapine (NVP), and lamivudine (3TC) - for mutagenicity in a bacterial reverse mutation assay. EFV and NVP are non-nucleoside reverse transcriptase inhibitors, AZT and 3TC are nucleoside reverse transcriptase inhibitors, and NFV is a protease inhibitor. All five chemicals were tested in a standard pre-incubation assay with *Salmonella typhimurium* strains TA98 and TA100, and *Escherichia coli* strain WP2 uvrA pKM101, with and without metabolic activation. Top concentrations tested varied by strain and metabolic activation condition and, in several cases, were limited by either toxicity or formation of precipitate. In the absence of limiting factors, the highest concentration tested was 6,000 ug/plate. No evidence of mutagenicity was seen with EFV, NFV, NVP, and 3TC under the conditions employed, while AZT gave a weak positive response, with and without S9, in the *E. coli* strain. In cases where results of previous mutagenicity testing are available, the results of the current assays are comparable. In summary, AZT, a mammalian cell mutagen and clastogen, was the only one of these five AIDS therapeutics to demonstrate mutagenic activity in bacteria, perhaps acting through attack at AT sites.

P121

Inhibition by Resistant Starch of Red Meat-Induced Promutagenic Adducts in Mouse Colon. Le Leu R[1,2], Winter J[1], Hu Y[1], Nyskohus L[1], Conlon M[2], Bird A[2], Topping D[2], Young G[1]. [1]Flinders University, Adelaide, SA, Australia, [2]CSIRO, Food and Nutritional Sciences, Adelaide, SA, Australia.

Introduction: Population studies have shown that high red meat intake may increase colorectal cancer risk. Our aim was to examine the effect of different amounts and sources of dietary protein on induction of the pro-mutagenic adduct O^6-Methyl-2-deoxyguanosine (O^6MeG) in colonocytes, to relate these to markers of large bowel protein fermentation and ascertain whether increasing colonic carbohydrate fermentation modified these effects. Methods: Mice (n=72) were fed 15% or 30% protein as casein or red meat, or 30% protein with 10% high amylose maize starch as the source of resistant starch. Genetic damage in distal colonocytes was detected by immunohistochemical staining for O^6MeG and apoptosis. Faeces were collected for measurement of pH, ammonia, phenols, p-cresol and short chain fatty acids. Results: O^6MeG and faecal p-cresol concentrations were significantly higher with red meat compared to casein ($p<0.018$), with adducts accumulating in cells at the crypt apex. DNA adducts ($p<0.01$) and apoptosis ($p<0.001$) were lower, and protein fermentation products (faecal ammonia, $p<0.05$ and phenol, $p<0.0001$) higher in mice fed resistant starch. Faecal short chain fatty acids levels were also higher in mice fed resistant starch ($p<0.0001$). Discussion: This is the first demonstration that a diet high in red meat can increase pro-mutagenic adducts (O^6MeG) in the colon. The delivery of fermentable carbohydrate to the colon (as resistant starch) appears to switch from fermentation of protein to that of carbohydrate and a reduction in adduct formation, supporting previous observations that dietary resistant starch opposes the mutagenic effects of dietary red meat.

P122

Tumor Necrosis Factor Receptors, Micronuclei Induction, Associated Adaptive Responses. Murley JS, Miller RS, Weichselbaum RR, Grdina DJ. The University of Chicago, Chicago, IL, United States.

Introduction: Adaptive processes that protect against radiation-induced micronuclei formation can be induced in cells by exposing them to low dose ionizing radiation (10 cGy) and to thiols such as amifostine, WR1065 (40 μM). While both processes culminate with elevated levels of manganese superoxide dismutase (SOD2) enzymatic activity, the tumor necrosis factor α (TNFα) signaling pathway is implicated in the low dose radiation- but not in the thiol-induced pathway. Methods: The goal of the study was to investigate the roles of TNFα receptors 1 and 2 (TNFR1,2) on the adaptive responses using micronuclei formation as an endpoint. BFS-1 wild type (WT) cells were exposed 24 h prior to a 2 Gy dose of ionizing radiation to either 10 cGy x-rays or a 40 μM dose of WR1065. BFS2C-SH02 defective in TNFR1 and BFS2C-SH22 defective in both TNFR1 and 2, confirmed by quantitative PCR, were exposed similarly. Results: A 10 cGy dose of radiation induced 24 h later a significant elevation of SOD2 activity in BFS-1 ($P<0.001$) and BFS2C-SH02 ($P<0.001$) but not BFS2C-SH22 ($P=0.433$). In contrast, WR1065 significantly induced SOD2 activity in all cell lines ($P<0.001$; $P<0.001$; and $P=0.02$; respectively). Elevated SOD2 activity correlated with significant protection against radiation-induced micronuclei formation ($P<0.004$). The adaptive effect was completely inhibited by transfection with SOD2 siRNA. Conclusion: Loss of both TNFR1 and 2, but not TNFR1 only, can abrogate the low dose radiation- but not the thiol-induced adaptive response mediated by SOD2. Supported by grants from DOE (DE-SC0001271) (D.J.G.) and NIH (R01-CA132998) (D.J.G.).

P123

Combination of Selenium and Green Tea Improves the Efficacy of Chemoprevention of Selenium and Green Tea Alone in a Rat Colon Cancer Model. Hu Y, Le Leu RK, Nyskohus LS, Winter J, Young GP. Flinders Centre for Cancer Prevention and Control, Flinders University, Adelaide, SA, Australia.

Introduction: The human colonic genome is constantly subjected to damage from environmental carcinogens present in food and tobacco. Therefore, factors that modulate apoptosis and DNA repair are likely to have profound effects on cancer risk. We have shown previously in azoxymethane (AOM) induced-colon cancer models that selenium (Se) enhance apoptosis and green tea extract increases DNA repair enzyme O^6-alkylguanine DNA alkyltransferase (MGMT). Aims: To determine if combination with pro-apoptotic agents and MGMT regulating agents improves the efficacy of chemoprevention. Methods: Four-week old rats (n=15/group) were treated with control diet, 0.5% green tea diet, 1ppm Se diet, and Se + green tea diet. After 2 weeks on diet, animals received 2 weekly AOM injections (15mg/kg) to induce aberrant crypt foci (ACF) formation. Animals remained on the same diet throughout the study until killed 8 weeks later. Colons were examined for ACF formation by 0.1% methylene blue. Cell proliferation (Ki-67), inflammation (COX-2), Wnt/ß-catanin pathway (ß-catanin) were also examined by immunohistochemical staining. Results: Se diet significantly inhibited ACF formation, compared to control diet, $p<0.05$; green tea diet also showed a trend of inhibition of ACF. The combination of Se and green tea significantly reduced all size of ACF, this effect was significant compared to each of Se or green tea diet alone, $p<0.05$. The combination also significantly inhibited cell proliferation, COX-2 and ß-catanin expression in the colon, compared to Se or green diet only diets, $p<0.05$. Conclusions: Combination of dietary agents with different mechanism of action provides better protection than either agent alone.

P124

Genotoxicity of Chemopreventive Agents. Doppalapudi RS[1], Riccio ES[1], Davis Z[1], Menda S[1], Du NN[1], Green CE[1], Kapetanovic IM[2]. [1]SRI International, Menlo Park, CA, United States, [2]National Cancer Institute, NIH, Bethesda, MD, United States.

The genotoxicity of 3 cancer chemopreventive drug candidates was evaluated. The candidates were 1) CP31398, a cell permeable styrylquinazoline, which is a p53 modulator; 2) SHetA2, a flexible heteroarotinoid; and 3) phospho-ibuprofen (PI), a novel derivative of ibuprofen. Ibuprofen and its moiety, diethoxyphosphoryloxybutyl alcohol (DEPBA) were also tested for chromosomal damage in comparison with PI. CP31398 and SHetA2 were both negative in all genetic toxicology tests used. PI was negative for *Salmonella-Escherichia coli*/microsome plate incorporation test (Ames test) and for the *in vivo* mouse bone marrow micronucleus test. In the *in vitro* chromosomal aberration assay using Chinese hamster ovary cells (CHO), PI was negative in the absence of S9; however it was positive in the presence of S9. PI was also positive for kinetochore negative (Kin-) and kinetochore positive micronuclei (Kin+) in the presence of S9 at noncytotoxic dose levels in the *in vitro* CHO micronucleus assay. Ibuprofen, a parent compound was negative for chromosomal aberrations, positive for micronuclei with Kin+ at the highest dose level and positive for micronuclei with Kin- at the two highest dose levels tested which may indicate that this drug induces specific types of aberrations. The moiety of ibuprofen, DEPBA, was negative for chromosomal damage in both the *in vitro* chromosomal aberration and micronucleus assays. PI, ibuprofen and DEPBA were non-genotoxic in the presence and absence of S9 in the Green Screen assay. The possible mechanisms of chromosomal damage by PI in the presence of S9 will be discussed. Supported by NCI Contract No.: N01-CN-43305.

P125

Intestinal Microbiota Reveals a Radio-Protective Effect on Genotoxic Endpoints upon Irradiation of Whole-Body Mice with High-Energy Protons. Maier I[1], Parfenova L[1], Yamamoto ML[2], Schiestl RH[1]. [1]Department of Environmental Health Sciences, School of Public Health, University of California, Los Angeles, CA, United States, [2]Rinat Neurology, Pfizer, South San Francisco, CA, United States.

Investigating the individual susceptibility of humans to radiation is influenced by large uncertainties resulting from radiation-quality, instabilities of dose-rates, and transfer of risk across populations. To elucidate the role of intestinal microbiota on acute and persistent radiation-induced DNA damage measured in blood, we used mice with conventional microflora (CM) and mice which have been re-derived into a semi-defined restricted (RM) microbiota (originally inoculated with only a few bacterial species). 10-14 week old mice were exposed to a total dose of 1 Gy from ionizing high-energy protons (2.5 GeV/n, LET = 2 keV/µm), as well as silicon (850 MeV/n, LET~ 50 keV/µm), and ^{56}Fe ions (1 GeV/n, LET = 150 keV/µm), radiation such as found in space. Blood collection was performed 6 hours post a single whole body exposure and DNA damage investigated by the comet assay. RM mice irradiated with high-energy protons had significantly higher levels of DNA damage compared with CM mice. Female mice showed a higher frequency of DNA strand breaks than male mice, with absolute mean Olive Tail Moments up to 20 for RM. In comparison, after exposure to silicon ions only few DNA strand breaks were observed, whereas irradiation with ^{56}Fe ions induced an overall general damaging effect on chromosomal DNA for both, all male and female mice. Since our preliminary results showed that RM mice had higher levels of DNA damage after exposure to accelerated protons compared to CM mice, we hypothesize that a fully developed conventional microbiota may have a protective effect.

P126

Genotoxicity of Styrene-Acrylonitrile Trimer in Brain, Liver, and Blood Cells of Weanling F344 Rats. Hobbs CA[1], Chhabra RS[3], Recio L[1], Winters J[1], Shepard K[1], Allen P[1], Streicker M[2], Witt KL[3]. [1]Genetic and Molecular Toxicology Division, ILS, Inc., Research Triangle Park, NC, United States, [2]Investigative Toxicology Division, ILS, Inc., Research Triangle Park, NC, United States, [3]National Toxicology Program, NIEHS, Research Triangle Park, NC, United States.

Styrene-acrylonitrile trimer (SAN Trimer), a byproduct in the production of acrylonitrile styrene plastics, was identified at a Superfund site in Dover Township, NJ, where childhood cancer incidence rates were elevated from 1979 - 1995. SAN Trimer was tested by the National Toxicology Program in a 2-yr perinatal carcinogenicity study in F344/N rats and a bacterial mutagenicity assay; results of both assays were negative, although a small number of rare CNS tumors was seen in dosed rats. To assess the potential for *in vivo* genotoxicity of SAN Trimer, a combined micronucleus (MN)/Comet assay was conducted in weanling male and female F344 rats. SAN trimer was administered by gavage (37.5, 75, 150, 300 mg/kg/day) once daily for 4 days; animals were sacrificed 4 hours after the last dosing. Micronucleated reticulocyte (MN-RET) frequencies in blood were determined by flow cytometry, and DNA damage in blood leukocytes, and liver and brain cells was assessed using the Comet assay. Highly significant dose-related increases (p < 0.0001) in MN-RET were measured in male and female rats administered SAN Trimer. Significant, dose-related increases in DNA damage (Comet assay) were measured in brain cells of both male (p < 0.0074) and female (p < 0.0001) rats; increased levels of DNA damage were also observed in liver cells and leukocytes. Chemical-related cytotoxicity was not indicated in the tissues examined for DNA damage. In summary, oral exposure of male and female F344 juvenile rats to SAN Trimer produced significant genetic damage in multiple tissues, as measured by a combined MN/comet assay.

P127

Genotoxic Effects of Antihypertensive Drug Atenolol. Kasper A, Gothke S, Dudley R, Vaglenov A. University of Findlay, Findlay, OH, United States.

Investigations of the hypertensive drug atenolol (CAS#29122687) have generated contradictory results for *in vitro* and *in vivo* genotoxicity. A positive micronuclei (MN) response on peripheral lymphocytes from patients chronically treated with atenolol has been reported (Telez *et al.* 2010), and a negative response after evaluation by sister chromatid exchange (Telez *et al.* 2000). The aims of this study are to evaluate the possible *in vitro* genotoxic effect of atenolol in presence of bleomycin on human peripheral blood lymphocytes. The genotoxic effect was detected through *in vitro* evaluation of DNA damage in human lymphocytes using the CBMN cytome and alkaline comet assays biomarkers: micronuclei, nuclear bridges, nuclear buds, apoptotic and necrotic cells, tail and head intensity, and olive moment. Atenolol was aded to give final concentration of 1, 10, 20, 40, 80, 160 and 320 mkg/ml. An increase in the frequency of MN after exposure to atenolol was observed when compared with control level. Statistical analysis in the dose-range of 1-20 mkg/ml gives us linear dependency from atenolol concentrations. Higher doses of 80, 160, and 320 mkg/ml are toxic. A similar increase has been found for NB yields. All other endpoints showed no statistical differences when compared with control. Comet assay slides analisys showed genotoxic effect after higher doses of atenolol only. This study revealed a mild genotoxic effect of atenolol on human lymphocytes after *in vitro* exposure.

P128

***LacZ* Containing Mouse Primary Hepatocytes are Valuable Tools in Genotoxic Risk Assessment.** Zwart PE[1], Schaap MM[1,2], van den Dungen MW[1], Braakhuis HW[1], White PA[3], van Steeg H[1,2], van Benthem J[1], Luijten M[1]. [1]National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands, [2]Department of Toxicogenetics, Leiden University Medical Center, Leiden, Netherlands, [3]Environmental Health Sciences and Research Bureau, Research and Radiation Directorate, Health Canada, Ottawa, ON, Canada.

Safety assessments of substances with regard to genotoxicity are generally based on a combination of *in vitro* and *in vivo* tests. These tests are performed according to a (tiered) test strategy whereby a positive result *in vitro* triggers further testing *in vivo*. The sensitivity of most *in vitro* mammalian cell genotoxicity assays is rather high, however, their specificity is often very low. This is the underlying cause of the remarkably high occurrence of irrelevant positive results. This high rate of misleading (positive) results *in vitro* is associated with an additional use of a high number of experimental animals, which is in many cases a waste of time and costs. Therefore, alternative *in vitro* assays that are more predictive for true *in vivo* genotoxicity are in demand. We investigated whether primary hepatocytes derived from lacZ carrying mice are a suitable alternatives. Proliferating primary hepatocytes were characterized in terms of biotransformation capacity and p53 functionality. Four genotoxic substances with clear different modes of action, *i.e.* benzo[a]pyrene, etoposide, bleomycin and cyclophosphamide, showed a concentration-dependent increase in mutant frequency. Our conclusion is that lacZ primary mouse hepatocytes are promising tools for the assessment of genotoxicity.

P129

***In Vivo* Exposure to Lipopolysaccharide (LPS), Granulocyte-Colony Stimulating Factor (G-CSF), and Gene Expression-Based Radiation Exposure Detection in Mice.** Tucker JD[1], Grever WE[1], Joiner MC[1], Konski AA[1], Thomas RA[1], Smolinski JM[1], Divine GW[2], Auner GW[1]. [1]Wayne State University, Detroit, MI, United States, [2]Henry Ford Hospital, Detroit, MI, United States.

In a large-scale nuclear incident thousands may be exposed to a wide range of radiation doses. Individualized rapid biodosimetry will be required to estimate exposures and make treatment decisions. Dosimetry may be assessed by qPCR because this method is fast, sensitive, reliable, and can be performed in the field. To ameliorate adverse effects of exposure, victims may be treated with cytokine growth factors including G-CSF, which stimulates granulopoiesis. Some victims will have thermal burns and other trauma that may cause systemic infections. The existence of infections and administration of G-CSF may confound the ability to achieve reliable dosimetry by gene expression analyses. Here, C57BL/6 mice were used to determine the extent to which G-CSF and LPS, which simulates infection by gram-negative bacteria, alter expression of genes that are either radiation-responsive or non-responsive; the latter have potential for use as endogenous controls. Mice were acutely exposed to cobalt-60 gamma rays at either 0 or 6 Gy, and injected 2 hours later with 0.1 mg/kg G-CSF, 0.3 mg/kg LPS, or vehicle. Expression levels of 96 different gene targets were evaluated in peripheral blood 4 or 24 hours later using qPCR. Expression levels of some genes were altered by LPS but generally not by G-CSF. Many genes therefore retain utility for biodosimetry or as endogenous controls, indicating that qPCR-based expression analyses may have utility in biodosimetry even in the presence of an infection or following G-CSF treatment. (Study funded by the Biomedical Advanced Research and Development Authority, US DHHS, under Contract HHSO100201000004C.)

P130

Skin Carcinogenesis in Mice Following Application of Individual Polycyclic Aromatic Hydrocarbons (PAHs) and Environmental Mixtures. Baird WM[1], Siddens LK[1], Krueger SK[1], Larkin A[1], Swanson HI[2], Arlt VM[3], Phillips DH[3], Löhr CV[4], Tilton SC[5], Waters KM[5], Williams DE[1]. [1]Environmental and Molecular Toxicology, Oregon State University, Corvallis, Oregon, United States, [2]College of Medicine, University of Kentucky, Lexington, Kentucky, United States, [3]Institute of Cancer Research, Sutton, London, United Kingdom, [4]Biomedical Sciences, Oregon State University, Corvallis, OR, United States, [5]Pacific Northwest National Laboratory, Corvallis, OR, United States.

PAHs have reemerged as pollutants of concern. As energy demands on a global scale continue to increase, human exposure to PAHs becomes a more serious health problem. PAHs form from incomplete combustion of organic material *e.g.* cigarette smoke, gasoline, and wood products. While benzo[a]pyrene (B[a]P) and dibenzo[def,p]chrysene (DBC) produce tumors in lung, skin, liver, and breast, the potency, target tissues, and mechanisms of action for environmental PAH mixtures are not well understood. A 2- stage mouse skin tumor model compared exposures to known PAHs and environmental mixtures. FVB/N mice were shaved and initiated with toluene vehicle, B[a]P, DBC, or 1 of 3 different PAH mixtures, followed by 25 weeks of promotion with phorbol 12-myristate 13-acetate (TPA). Skin tumors were harvested, fixed, H&E stained, and analyzed for progression from papilloma to carcinoma. DNA adducts were measured 12 hours post initiation using 32P-post labeling to correlate adduct formation with subsequent tumor response. Gene expression levels were measured by microarray in dermal samples collected 12 hours post-initiation. Skin tumor incidence, multiplicity, and progression to carcinoma were all highest in the DBC treated mice. B[a]P, and two of the mixtures produced similar results in all parameters measured with exception of progression to carcinomas. Gene expression changes in the positive controls, including AhR-mediated upregulation of Cyp1a1 in B[a]P and upregulation of Cyp1b1 in DBC, were identified and utilized as signatures of PAH-mediated perturbation of the gene transcriptional network. Transcriptional changes observed in the mixtures were examined for these signatures. Study supported by USPHS grant P42_ES016465.

P131

Tissue-Specific Effect of Peroxiredoxin 1 Deficiency on *In Vivo* Loss of Heterozygosity Mutations in Mice. Rani V[1], Neumann C[2], Shao C[1], Tischfield J[1]. [1]Department of Genetics, Rutgers University, Piscataway, NJ, United States, [2]Department of Cell and Molecular Pharmacology and Experimental Therapeutics, Medical University of South Carolina, Charleston, SC, United States.

The loss of the H_2O_2 scavenger Prdx1 in mice leads to an elevation of oxidative stress, oxidative DNA lesions and a predisposition to tumorigenesis. Loss of heterozygosity (LOH) mutations has been observed to promote cancer when they involve loci of tumor suppressor genes. A connection between oxidative stress and LOH mutations *in vivo* has not yet been established. In this study, we observed that the severity of reactive oxygen species (ROS) is much higher in fibroblasts than in T-cells, and that the level of ROS was higher in both fibroblasts and T-cells derived from $Prdx1^{-/-}$ mice compared to $Prdx1^{+/+}$ mice. Using Aprt as a reporter, we characterized the LOH mutants in fibroblasts and splenic T-cells of Prdx1 deficient adult mice and observed an elevation in LOH mutant frequency in fibroblasts, but not in T-cells, of $Prdx1^{-/-}$ mice. Examination of the expression of other genes whose products are involved in ROS scavenging revealed no significant difference between $Prdx1^{-/-}$ mice and $Prdx1^{+/+}$ mice. However, the expression of a mismatch repair gene, Mlh1, was significantly downregulated in fibroblasts, but not in T-cells, of $Prdx1^{-/-}$ mice. Therefore, the combination of elevated oxidative stress and a down-regulation of a DNA repair gene known to be involved in recombination may contribute to the elevation of LOH in fibroblasts of $Prdx1^{-/-}$ mice. We conclude that each tissue may have a distinct mechanism through which Prdx1 deficiency promotes tumorigenesis.

P132

HVRII of mtDNA in Cord Blood Cells of Newborn Children and in Their Saliva 10 Years Later. Schmuczerova J[1], Torokova P[2], Topinka J[1], Dostal M[1], Sram RJ[1], Brdicka R[1]. [1]Institute of Experimental Medicine AS CR, Prague, Czech Republic, [2]Charles University in Prague, Faculty of Science, Prague, Czech Republic, [3]Institute of Haematology and Blood Transfusion, Prague, Czech Republic.

Many polymorphisms have been described in the mtDNA, and most accumulate in the noncoding regulatory region or displacement loop (D-loop). The D-loop is the location of mitochondrial transcription promoters. It is the most important control region for the regulation of the genetic function of human mtDNA. The role of D - loop mtDNA polymorphisms in the etiology and pathology of various diseases has been the focus of many studies. In this study we compared hypervariable region II nucleotide sequences of mtDNA obtained from cord blood cells and saliva cells of the same individual at birth and after ten years at the so called mutation hot spots. Cord blood and saliva samples used in this study were obtained from 245 children living in two areas of the Czech Republic (the industrial and agricultural districts). Cord blood samples were collected within the period of 1994-1998, and saliva samples were collected at the age of children between 8-12 years. The personal identity of samples was proved by short tandem repeat profiling. Comparison of observed sequences with the sequence mentioned at http://www.mitomap.org/MITOMAP/PolymorphismsControl proved that all "deviations" from the "standard" were already observed and included in haplogroups identified in the Czech population with the exception of nucleotides Nr.243 (transition), 291, 296 and 299 (transversions). Comparison of individuals living in two regions differing by the extent of the air pollution, however, did not reveal significantly increased number of mutations in the population living in the polluted region. Supported by the Czech Ministry of the Education (Grant#2B08005).

P133

Toxicity of Anti-HIV Drugs and the Use of Tempol As a Therapeutic Agent. Nguyen PG[1,2], Liu YM[1], Poirier MC[1]. [1]National Institutes of Health, Bethesda, MD, United States, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States.

NRTIs are essential components of the successful antiretroviral combination therapies used for treatment of HIV-1. However, during long-term therapy NRTIs damage heart mitochondria, thus limiting clinical use of these drugs. Consequently, reducing NRTI-induced mitochondrial toxicity may benefit HIV-1-infected patients. In these studies we have used H9c2 rat cardiomyocyte cultures to examine mitochondrial toxicity induced during long-term NRTI exposure. To evaluate intervention we have used the stable free radical Tempol and its metabolite Tempol-H. These compounds are cyclic nitroxides with antioxidant properties. H9c2 cells were cultured for 4 passages in the presence of 150 μM Zidovudine (AZT) and 150 μM Didanosine (ddI), and cytotoxicity was measured by Incucyte in the presence and absence of 100 μM Tempol and 100 μM of its metabolite Tempol-H. Maximal oxidative capacity was evaluated by Seahorse XF24, which measures oxygen consumption rate (OCR). At 48 hr of growth, for cells exposed for 4 passages, the cell survival for cells treated either with AZT/ddI or AZT/ddI plus Tempol or Tempol-H was 50% of that in the unexposed controls; addition of Tempol or Tempol-H did not restore cell survival. At passage 4 the maximal uncoupled OCR of cells exposed to AZT/ddI was about 50% of the unexposed controls, and incubation with Tempol or Tempol-H restored the maximal uncoupled OCR capacity to the level of the unexposed controls. The extent of restoration was statistically significant ($p<0.05$). Currently, we are evaluating underlying mechanisms related to the effect of Tempol and Tempol-H as mitochondrial protective agents.

P134

Evaluation of Propylene Oxide (PO)-Induced Mutagenicity in a p53-Competent, Human-Derived Cell Line, TK6. Schisler MR[1], Gollapudi BB[1], Moore NP[2], Pottenger LH[1]. [1]The Dow Chemical Company, Midland, MI, United States, [2]Dow Europe, Horgen, Switzerland.

Chronic inhalation exposure to high levels of propylene oxide (PO; CAS 75-56-9) induces site-of-contact nasal tumors in rodents. Extensive data supports a threshold mode-of-action (MOA) for tumor induction, with severe, sustained GSH depletion associated with increased cell proliferation in target nasal respiratory epithelium as initial key events. While PO is directly DNA-reactive, it is unclear whether this activity has any role in the early events of tumorigenesis. Characterization of the dose-response for PO-induced mutations will provide perspective on any relevance for PO-induced mutagenicity in the threshold cancer MOA. This report describes the determination and validation of experimental conditions to detect PO-induced mutations at the HPRT and TK loci in the human-derived TK6 cell line, which has a functional p53 gene, using ethyl methanesulfonate and N-nitroso-N-ethylurea as positive controls. The direct mutagenic potential of PO was assessed in two independent assays with PO concentrations from 0.1 to 100 mg/ml, based on PO cytotoxicity. Statistically significant increases in induced mutant frequencies (MF) were observed at concentrations >6.25 and >25 mg /ml for the TK and HPRT loci, respectively. Based upon reproducible and statistically significant increases in MF in TK6 cells, PO was considered capable of eliciting a mutagenic response. Eventual determination of a No-Observed-Genotoxic-Effect-Level (NOGEL) for PO induction of gene mutations in TK6 cells will further contribute to the evidence for a practical threshold for PO-induced tumor induction at the site-of-contact tissue. These data add to the growing weight-of-evidence for the existence of thresholds for mutation induction by DNA-reactive chemicals.

P135

Does the Presence of Rodent S9 Liver Extract Contribute to the Prevalence of Irrelevant *In Vitro* Mammalian Genotoxicity Data? Tate MJ[1,2], Walmsley RM[1]. [1]Gentronix Ltd, Manchester, United Kingdom, [2]University of Manchester, Manchester, United Kingdom.

First pioneered in the Ames test, *in vitro* genotoxicity testing relies on the inclusion of exogenous metabolism, usually in the form of rodent S9 fraction, to allow the assessment of possibly genotoxic metabolites. However, no systematic data analysis has been undertaken to discover whether this inclusion affects the specificity of *in vitro* mammalian genotoxicity tests. This study reports such an analysis. Ames negative compounds with positive *in vitro* mammalian test data were selected from the data collated by Kirkland *et al.* 2005. Amongst these, 89 compounds were identified which were positive in at least one regulatory *in vitro* mammalian test in the presence of S9. 51 of the 89 were readily available: 28/51 were non-carcinogens and 23/51 rodent carcinogens. These 51 were tested with the GADD45a-GFP *in vitro* mammalian genotoxicity assay in the presence and absence of S9: the assay is expected to produce positive results for carcinogens with mutagenic, aneugenic and clastogenic mechanisms of action. 8/51 produced positive GADD45a-GFP assay results. Of the 23 rodent carcinogens tested, 8 are *in vivo* negatives and 16 only generated tumours in rats or mice at single foci. Further analysis will be presented. In summary, the data from both the literature survey and the GADD45a-GFP assay suggest that the inclusion of exogenous metabolism into *in vitro* mammalian genotoxicity assays does not lead to an increase in positive results. S9 itself is not a confounding factor of poor specificity in the *in vitro* mammalian assays.

P136

***In Vitro* Genotoxicity Tests Using Primary Hepatocytes.** Horibata K, Ukai A, Masumura K, Nohmi T, Honma M. National Institute of Health Sciences, Setagaya-ku, Tokyo, Japan.

Introduction: *In vitro* genotoxicity assays provide an opportunity for high-throughput analyses of test chemicals, but have a limited endogenous capacity to metabolize them. However, cultured primary hepatocytes retain the characteristics of liver cells and have been shown to contain a broad spectrum of metabolizing enzymes. To develop and to form the basis *in vitro* genotoxicity assays with hepatocytes, we performed gpt mutation assay using primary hepatocytes. Methods: Primary hepatocytes were isolated from gpt delta transgenic mice by an adaptation of a two-step collagenase perfusion technique that involves enrichment prior to culturing using Percoll isodensity purification. To confirm DNA replication in primary hepatocyte under epidermal growth factor (EGF) and hepatocyte growth factor (HGF) treatments, we analyzed incorporation of 5-ethynyl-2'-deoxyuridine under these conditions. gpt mutation assay was performed as previously reported. Results: Transgene gpt mutant frequency clearly increased in benzo[a]pyrene and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) treated primary hepatocytes cultured in the presence of EGF and HGF. Additionally, nascent DNA synthesis was also clearly observed in our conditions. Discussion: Our results indicated that this *in vitro* genotoxicity assay using primary hepatocytes was useful to assess the genotoxic activity of test chemicals requiring metabolic activation by cytochrome P450 isozymes and other enzymes. Additionally, it is expected that this *in vitro* method could be applied to high-throughput analyses and to analyses for genotoxicity of test chemicals, *e.g.*, acrylamide, which are difficult to assess genotoxicity because of the potent toxicity *in vivo*.

P137

A Summary of *In Vitro* Genetic Toxicology Assay Results: The Rest of the Story. Clarke JJ, Madraymootoo W, Wagner VO, Pant K, Young RR, Aardema MJ. BioReliance, Rockville, MD, United States.

Changes to *in vitro* genetic toxicology testing over the last 5 years include: lead optimization approaches like SAR, screening assays to identify and eliminate potentially genotoxic chemicals from further consideration, better guidance on cytotoxicity assessment and dose selection, and greater use of p53-competent human cells. We examined the pattern of positive results across assays conducted by BioReliance from 2005 through 2010. Results were tabulated for GLP-compliant Ames, mouse lymphoma (MLA), chromosome aberration in CHO and human peripheral blood lymphocytes (HPBL) assays along with non-GLP screening Ames assays. There was a decrease in positive results in MLA and CHO chromosome aberration assays while the rate for Ames assays stayed constant. The decreases may be due to the factors stated above. In addition, the decrease in positive results in the MLA may be due to establishment of the Global Evaluation Factor in 2003 and in chromosome aberration assays due to use of relative increase in cell count as a measure of cytotoxicity. BioReliance has seen an increase in the number of screening studies (Green Screen, Ames II, and Ames screening assays); however, the percent positive results in Ames screening assays has remained unchanged over the last few years. There has been an increase in the % positive results in the chromosome aberration assay in HPBL. This may be due to the use of this assay as a follow-up to positive CHO chromosome aberration assays and its generally increased use with the resultant increase in the number of assays with positive results.

P138

Miniaturization of a *Salmonella Typhimurium* Genotoxicity Assay. Elespuru RK[1], Rajani A[1,2]. [1]U.S Food and Drug Administration, Silver Spring, MD, United States, [2]University of Maryland, Baltimore County, Baltimore, MD, United States.

The standard bacterial mutagenicity (Ames) assay is used world wide to test for genotoxicity of medical devices, drugs, and other products. Generally, 100µl of test substance is used per assay (x 5 strains in triplicate). There is a great need for alternative methods that use less sample, *e.g.* for tests of extracts of small medical devices. We have experimented with a hybrid method, using pre-incubation in microtiter dishes for sample exposure, followed by plating as for the standard assay. Pre-incubation may increase sensitivity because the bacteria are incubated directly with the sample, resulting in a higher effective concentration. We examined whether we could detect one tenth volume of sample using pre-incubation. *Salmonella* TA100 (100µl) and EMS, MMS, or DMSO solvent (10µl) were added to wells in a 96 well microtiter plate. The microtiter plates were incubated at 37degrees C and the well contents removed and plated after times varying from 0 to 120 min. The solvent controls were plated at zero time and at the last incubation point. Standard assays for EMS and MMS were conducted for comparison. Pre-incubation of bacteria with EMS led to a time-dependent sensitivity enhancement near 100-fold at 120min. This surprising result was not replicated with MMS, which exhibited a more modest enhancement in mutant yield as a result of pre-incubation. Pre-incubation with DMSO did not appear to be toxic to the bacteria or increase the background appreciably. The pre-incubation protocol appears worthy of further investigation as a means of maintaining assay sensitivity while using less sample.

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Analysis of *In Vitro* Chromosomal Aberration Test Data with CHL Cells for Reducing Top Test Concentration for Industrial Chemicals. Morita T[1], Honma M[2], Morikawa K[1]. [1]National Institute of Health Sciences, Division of Safety Information on Drugs, Food and Chemicals, Tokyo, Japan, [2]National Institute of Health Sciences, Division of Mutagenesis and Genetics, Tokyo, Japan.

A current concern with *in vitro* mammalian cell genotoxicity testing is the false or misleading positive results caused by the use of excessively high test concentrations. A dataset of 249 industrial chemicals used in Japan was analyzed. Of those, 116 (46.6%) were positive in the *in vitro* chromosomal aberration (CA) test, including 6 that were positive only at >10 mM. There were 59 CA-positive chemicals at ≤1 mM. At >1 mM, 51 chemicals were CA-positive, including 13 Ames-positive chemicals which were not "missed" by the test battery. Thus, 38 potentially positive chemicals would have been missed if the top test concentration were 1 mM. Analysis of the relevance of CA results on the 38 missed chemicals was conducted based on weight of evidence approach, including evaluations of effects of extreme culture conditions (low pH, high toxicity, or precipitation), *in silico* structural alert analysis, *in vivo* genotoxicity and carcinogenicity test data, mode of action, or information from closely related chemicals. Among the 38 missed chemicals, 12 or remaining 26 were considered minimal/some concern or negligible concern for human health risk assessment, respectively. If several top concentration limits were applied to the 38 missed chemicals, 2 mM or 1 mg/mL, whichever is higher is the most effective, *i.e.*, relatively higher (8/12) or lower (17/26) detection among 12 or 26 chemicals, respectively. Therefore, we propose 2 mM or 1 mg/mL, whichever is higher as the top concentration limit for industrial chemicals.

P140

Deregulated Genes and Pathways in Human Embryonic Lung Fibroblasts (HEL 12469) treated with Complex Mixtures of the Air Pollutants. Topinka J[1], Libalova H[1], Hanzalova K[1], Klema J[2], Sram RJ[1]. [1]Institute of Experimental Medicine AS CR, Prague, Czech Republic, [2]Czech Technical University in Prague, Prague, Czech Republic.

Recently, we have demonstrated the genotoxic activities of complex mixtures of organic extracts from urban air particles collected in various localities of the Czech Republic that differ in the extent and sources of air pollution [Topinka *et al.*, *Toxicology Lett.* 202 (2011) 186-192]. In this study, human embryonic lung fibroblasts (HEL12469) were employed to assess changes in gene expression profiles induced by complex mixtures of organic air pollutants adsorbed onto respirable air particles (PM2.5) collected by high volume samplers in 4 localities of the Czech Republic (Ostrava-Bartovice, Ostrava-Poruba, Karvina and Trebon). For this purpose, changes in the genome-wide expression profiles induced by extractable organic matter (EOM) from the PM2.5 particles were analyzed at subtoxic EOM concentrations of 10, 30, and 60 µg EOM/ml. Dose dependent increases of a number of deregulated genes were observed for all EOMs. Multiple transcripts/genes are significantly correlating with B[a]P content in EOM and multiple deregulated biological pathways were identified. The expression of selected deregulated genes from various biological pathways were verified by RT PCR. Pathways deregulated in all 4 localities include: metabolism of xenobiotics by cytochrome P450, wnt signaling, ABC transporters, glutathione metabolism, TGF-beta signaling and many others. The results suggest the complexity of biological effects induced by complex mixtures of organic air pollutants, and show that various epigenetic effects together with expected genotoxicity should be taken into the consideraton. Supported by the Ministry of the Education, Youth, and Sports of the Czech Republic (Grant #2B08005).

P141

Screening Genetic Toxicology Assays for Potential Genotoxic Impurities. Registre M, Hamel A, Dennis SJ. Charles River Laboratories Preclinical Services Montréal, Senneville, QC, Canada.

Genetic Toxicology assessment is required for observed levels of impurities exceeding relevant ICH qualification threshold or displaying a structural alert for mutagenicity. Regulatory agencies normally require a minimal series of *in vitro* genotoxicity tests that include the bacterial mutation and chromosome aberration tests to assess potential human health hazards. Upcoming revisions to ICH guidance also consider acceptability of the *in vitro* micronucleus test as an alternate mammalian cell assay. The preference of both the FDA and EMEA is for those assays to be conducted with the isolated impurity rather than the spiked API because of increased sensitivity. Considering the target high dose of 5mg per plate for the Ames assay or up to 5 mg/mL for the mammalian cell-based assays, the minimum of 1g needed to conduct the full GLP versions of these may be challenging. In an attempt to address these concerns and other issues related to limited availability of compounds, we have recently incorporated in our service offering the miniature versions of the Ames and in-vitro micronucleus test. Compound requirements for these range from 15-30 mg. The present data compares sensitivity of these scaled-down versions with the standard versions using known genotoxic compounds. Although not considered regulatory compliant, these may be acceptable as adjunct to full-sized AMES on the API/ Impurity mixture as part of a 'weight of evidence' approach to qualify the impurity. Additionally, we present data for our miniaturized, regulatory compliant chromosome aberration assay which can be performed with less than 75 mg of compound.

P142

Proposal for Class-Specific TTC Values of Potentially Genotoxic Impurities (PGI) in Pharmaceuticals. Zeller A, Brigo A, Struwe M, Singer T, Gocke E. F. Hoffmann-La Roche, Ltd., Basel, Switzerland.

The generic Threshold of Toxicological Concern (TTC) concept has been used to define acceptable levels of genotoxic impurities in pharmaceuticals. With the exception of carcinogens belonging to the group called Cohort of Concern, a dose of 1.5µg/day is generally accepted based on the calculation that only 30 out of the 528 non-CoC carcinogens reported by Kroes *et al.*, in 2004 would elicit more than 1 cancer case in 10 000 individuals exposed to such dose. The possibility to derive more specific safety factors for classes of substances with similar genotoxic modes of action was investigated using different approaches. Our analysis aims at refining the risk assessment for trace amounts of alkyl halides and aromatic amines, which are important classes of synthetic intermediates, as well as for a recent case example of a nitrosamine. The dataset of Kroes 2004 was clustered into several subsets according to their chemical structures and evaluated for specific relationships with their TD_{50} values. Additionally, the reported correlation between TD_{50} values and potencies in the Ames test was considered for aromatic amines. SAR analysis developed for monofunctional alkyl halides indicated that a 10-fold increase of the TTC level is justified. The same analysis conducted on a particular group of N-nitroso compounds points towards a 10-fold decrease of the standard TTC limit. No useful SAR could be derived for aromatic amines, but a reasonable correlation between their mutagenic potency and TD_{50} values was found and subsequently used to establish a maximum allowed concentration of specific aromatic amines as PGI.

P143

Predicting *In Vivo* Genotoxicity Using *In Vitro* Structural Alerts. Canipa S[1], Cayley A[1], Hamada S[3], Hirose A[2], Honma M[2], Williams R[1]. [1]Lhasa Limited, Leeds, United Kingdom, [2]National Institute of Health Sciences, Tokyo, Japan, [3]Mammalian Mutagenesis Study Group, Ibaragi, Japan.

While the *in vivo* genotoxicity of a compound may not always be well correlated with its activity in *in vitro* test systems, for certain compound classes a good overlap can exist between the two endpoints. In these cases, *in vitro* and *in vivo* activity may be described and predicted by a common structure-activity relationship. The difficulty, however, lies in establishing the cases for which this relationship holds true. With this in mind, a project was initiated in which existing structural alerts for *in vitro* chromosome damage in the expert system Derek for Windows (DfW) were assessed for their relevance to *in vivo* activity. Initially, the predictive performance of each *in vitro* alert was assessed against *in vivo* data, taken from the *in vivo* micronucleus and chromosome aberration tests. An expert assessment was then made regarding the relevance of the *in vitro* alert to *in vivo* activity and if appropriate the alert was extended to predict both endpoints. Data sources included a data set of micronucleus test results collated by the Mammalian Mutagenesis Study Group (MMS) in Japan, along with the Vitic Nexus and Leadscope Enterprise databases. A total of 17 *in vitro* alerts were investigated. Of these, 8 alerts were considered to have relevance *in vivo* and were extended. In addition, 1 new *in vivo* alert was implemented. These modifications led to a significant improvement in the coverage of *in vivo* chromosome damage in DfW, as measured against the MMS data set.

P144

Providing a Single Point of Access to Genetic Toxicity Models and Data. Myatt GJ[1], Bower D[1], Cross K[1], Crump M[1], Miller S[1], Saiakhov R[3], Tice RR[2]. [1]Leadscope, Inc., Columbus, OH, United States, [2]National Institute for Environmental Health Sciences, Research Triangle Park, NC, United States, [3]MultiCASE, Inc., Cleveland, OH, United States.

This poster describes a prototype of an integrated prediction system that brings together toxicity data and predictions within a single graphical user interface. This interface was developed to run within a web browser on any operating system using Web2.0 technology. The user can specify one or more query molecules by name or id as well as through structure searching options including exact structure searching, substructure searching and similarity structure searching. Alternatively, new chemical structures can be loaded directly. The user interface provides access to toxicology studies from multiple databases, including integrated genetic toxicity data. The user is able to assess the toxicity of a compound using QSAR models or structural alerts from multiple suppliers. The user interface supports restrictions based on toxicological endpoints of interest (*e.g.*, carcinogenicity, genotoxicity, reproductive toxicity, and so on). The system was designed for use by scientists with different backgrounds; hence, it has to be easy to use and transparent to all stake holders. The poster will illustrate use cases for profiling a set of compounds based on known genetic toxicity information, browsing genetox prediction model results from multiple vendors and understanding associations between rodent carcinogenicity and genetic toxicity results.

P145

Characterization of Thyroid Response Elements Identified Using a Toxicogenomics-Bioinformatics Screen. Paquette M[1], Dong H[2], Gagne R[2], Malowany M[2], Williams A[2], Wade M[2], Yauk C[2,1]. [1]Carleton University, Ottawa, ON, Canada, [2]Health Canada, Ottawa, ON, Canada.

Toxicant-induced disruption of thyroid hormone (TH) signalling can alter growth, development and energy metabolism leading to persistent mental and physical deficits. THs exert their effects through interactions with thyroid response element (TRE)-bound TH receptors, thereby controlling target gene transcription. However, only a handful of genes are known to be directly regulated by THs. The objectives of this research are to identify: (a) genes involved in TH response during liver development; and (b) candidate biomarkers for the study of TH-disrupting chemicals. Developmental hypothyroidism was induced in mice by treatment with methimazole and perchlorate from postnatal day 12 -15; a subset of these mice was supplemented with TH. A final group of mice was treated with TH prior to sacrifice. DNA microarrays were used to assess transcriptional response of these mice compared to euthyroid controls. Approximately 400 genes were altered in at least one treatment condition. An *in silico* search for TREs was performed on the promoter regions of responsive genes using a position weight matrix of known TREs coupled with a novel scoring system. TREs were identified in a subset of genes suggesting direct regulation by THs. TREs in four genes (hectd3, slc25a45, tor1a and 2310003H01Rik) were validated by chromatin immunoprecipitation and electrophoretic mobility shift assays. Thus, the research identified new genes that are under the direct control of TH *via* functional TREs. Characterization of thyroid receptor binding sites provides the basis for developing a more precise TRE sequence motif, and can be used to develop bioassays for TH disrupting chemicals.

P146

Environmental and Molecular Origin: A Comparison of Themorphology of Red Rain Samples from India and UK. Alharbi S. Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia.

Large quantities of red rain fell in parts of India in 2003. It has been variously described as fat globules, sand, and red blood cells and the alga Trentepohlia. Claims have also been made that red rain is cellular, but lacks DNA and that it has an extraterrestrial origin. A red rain event also recently occurred in the UK, thereby providing an opportunity to study red rain from two distinct sources. Here the similarities and differences between the cells occurring in the red rain events are discussed. The results show that different types of red rain exist. Key words: Red rain, astrobiology, aerobiology, exobiology.

P147

Bioaccumulation of Ag, Co and Ni by the Organs of *Typha domingensis* (Pers.) Poir. ex Steud. in Lake Burullus and Their Potential Use As Contamination Indicators. Alatar AA[1], Eid EM[2], El-Sheikh MA[1,3]. [1]Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia, [2]Department of Botany, Faculty of Science, Kafr El-Sheikh University, Kafr El-Sheikh, Egypt, [3]Botany Department, Faculty of Science, Damanhour University, Damanhour, Egypt.

The concentrations of Ag, Co and Ni in organs of *Typha domingensis* and their surrounding sediments from Lake Burullus, Egypt, were investigated monthly from February to September 2010 to evaluate the aquatic environment quality of the study area, and to test the suitability of its organs for bio-monitoring of sediment metals. The heavy metals of sediment were found to decrease in the order of Ni > Co > Ag. The concentrations of sediment heavy metals had no significant differences over time. In the present study, the sediment contents of Ag were 45 times above the worldwide range. On other hand, Co concentrations were below the reference ranges of US and Chinese soils. The bioaccumulation decreased according to the order of rhizome > root > leaf for Ag; and root > rhizome > leaf for Co and Ni. The transfer factors of Ag, Co and Ni from sediment to below-ground organs were smaller than one. Co had the maximum transport from below-ground to above-ground organs, while Ag had the minimum. There was a significant linear correlation between the concentration of Ag in root of *T. domingensis* and that in sediment. This result suggested the *T. domingensis* can be regarded as biomonitors on the Ag pollution of Lake Burullus.

P148

The Stability of the Bacterial Chromosome As a Model for Environmental Toxicology. Camacho-Carranza R, Hernandez-Guadarrama B, Hernandez-Ojeda SL, Espinosa-Aguirre JJ. Universidad Nacional Autónoma de México, México, DF, Mexico.

Several DNA repair mechanisms have been described, but little is known of the systems that prevent chromosomal rearrangements, associated to the loss of genomic stability. To explore the mechanisms of stability, we used *Salmonella enterica* serovar Typhimurium as a model. *Salmonella* and *Escherichia coli* derived from a common ancestor that lived 150 million years ago, and despite their DNA sequences have diverged significantly (10-20%) and its recombinogenic mechanisms are active, they share genome synteny, suggesting the presence of stability mechanisms. *Salmonella's* chromosomal his-nadA interval is permissive for inversion, but not the his-trp, however, mutation in the tus gene makes this interval permissive. Tus protein binds the "ter" sites. We believe that inside these mechanisms underlie general phenomena of genomic stability, with relevant implications to the area of environmental toxicology. Here we present the identification of three loci involved in the stability of his-trp segment, whose mutation ameliorate the inversion restriction. The loci are close to ydgT and yafE ORF's, and the gene fliK. yafE encodes a methyltransferase of the menaquinone synthesis, and is involved on the flagellum formation on anaerobic growth conditions; fliK controls the length of the flagellum, and ydgT is a paralog of hha in *E. coli*, a gene involved in the expression of several genes and forms complex with H-NS. It is the first time that these ORF's are reported to be involved in recombinogenic processes, therefore, to understand their role in chromosomal rearrangements we need to assess their participation in different recombinogenic pathways.

P149

Construction and Genetic Evaluation of Stable Bacterial Biocontrol Agent *via* Protoplast Fusion. Hassan AA, Sayed RM, Ibrahim EE, Sabit HH, Ibrahim SA. Misr University for Science & Technology, 6 of October City, Egypt.

A successful protoplast fusion between Serratia marcescens and Pseudomonas fluorescens was carried out. The stable fusant (designated Serratomonas) has combined the features of both strains. Evaluation of the obtained fusant as well as the two parents as biocontrol agent against nematodes and soil born diseases was performed. The biochemical characterization of the fusant was evaluated using HPLC. Molecular characterization of the fusant was carried out using Random Amplified Polymorphic DNA (RAPD), 16S rRNA and Restriction Fragment Length Polymorphism (RFLP). The results showed that the constructed fusant has gained its genetic makeup from both parents despite it was nearly related to the parent Pseudomonas fluorescens. Meanwhile the Biocontrol potentiality of the fusant was synergized in comparison to each parent individually.

P150

Environmental Pollution and Risk of Birth Defects in a South Indian Population from Andhra Pradesh. Jyothy A[1], Prasoona R[1], Sunitha T[1], Venkateshwari A[1], Anjana M[1], Shilpa Reddy G[1], Kumari TM[2], Sujatha M[1]. [1]Institute of Genetics & Hospital for Genetic Diseases, Hyderabad, Andhra Pradesh, India, [2]Government Maternity Hospital, Hyderabad, Andhra Pradesh, India.

Background: Birth defects are structural or functional abnormalities present at birth that cause physical or mental disability. Currently, birth defects are the leading cause of death for infants during the first year of life. Every year an estimated 8 million children - about 6 percent of total births worldwide are born with a serious birth defect of genetic or partially genetic origin. Human pregnancy and development is vulnerable to environmental exposures of the father and mother to chemical, biological and physical agents. Exposures associated with adverse developmental outcomes include; air and water pollution, chemicals in foods, occupational exposures, agricultural chemicals, metals, persistent and volatile organics. Methods: Six hundred high risk pregnancy cases were screened for birth defects using ultrasonography. Blood samples were collected in vaccutainers from high risk mothers and subjected to double and triple marker tests. Chromosomal aberrations were analyzed by conventional karyotyping and confirmed by FISH. Results: Preliminary results show that parents exposed to environmental pollution are at a high risk of producing children with birth defects. The most common defects were because of abnormalities of the CNS. Some of the birth defects were on account of chromosomal anomalies. Discussion: Environmental exposure to potential teratogens before and during early pregnancy (*i.e.* pre conceptional and prenatal) should be avoided to prevent congenital anomalies. Developmental defects which are linked with these exposures include; growth retardation, structural abnormalities and death.

P151

Genotoxic and Environmental Effects of the Implementation of Good Agricultural Practices in Colombian Tomato Fields. Narváez DM[1], Robayo A[1], Díaz S[2], Castro R[2], Páez MI[2], Varona M[2], Groot de Restrepo H[1]. [1]Universidad de los Andes, Bogotá, Colombia, [2]Instituto Nacional de Salud, Bogotá, Colombia.

Chronic exposure to pesticides has been associated to different health problems like cancer and neurotoxicity. But in Colombia, farmers are not always aware of the health risk that exposure to pesticides have and do not take the appropriate precautions. Furthermore, the most frequently used pesticides are organochlorines, organophosphates and carbamates which are also the most toxic. The aim of this study was to evaluate the effect of the implementation of good agricultural practices on DNA damage caused by pesticides in workers of tomato fields in Colombia. To achieve this, pesticide levels were measured from the environment and the micronuclei test was performed from peripheral lymphocytes of farmers before and after training in good agricultural practices. Results showed no differences between the micronuclei frequency before and after the intervention ($p=0.906$ and $p=0,750$, respectively). Nonetheless, pesticide levels in the environment, nucleoplamic bridge frequency and nuclear division index were significantly higher ($p<0.05$) before the training. In this case, we can conclude that there is a toxic effect of pesticides in DNA that could be diminished if working with good practices.

P152

Phenotypic Plasticity of Cardiomyopathy Based on Sarcomeric Gene Variations. Pratibha N[1], Satyanarayana ML[1], Deepa S[2], Advithi R[1], Narsimhan C[3]. [1]Department of Genetics, Osmania University, Hyderabad, India, [2]Centre for Cellular & Molecular Biology, Hyderabad, India, [3]Care Hospital, Hyderabad, India.

Cardiomyopathy is the disease of the heart muscle associated with which is caused by abnormalities in cardiac wall thickness, chamber size, contraction and relaxation. Hypertrophic cardiomyopathy (HCM) is known to be manifested by mutations in 12 sarcomeric genes and dilated cardiomyopathy (DCM) is linked to by cytoskeletal gene mutations. It is well established that phenotypic plasticity accounts forto both HCM and DCM phenotypes. The present study was an attempt to compare the genetic variations of sarcomeric genes viz., beta-myosin heavy chain gene (b-MYH7), Troponin T2 (TNNT2), Troponin I3 (TNNT3), Alpha - Tropomyosin (TPM1), Alpha- Actin (ACTC), Myosin Regulatory Light Chain (MYL2), Myosin Essential Light Chain (MYL3) which were screened by SSCP & sequencing in 179 controls, 309 patients (HCM & DCM). Genetic variations were observed in Exons 7, 12, 19 and 20 of MYH7, Exon 8 and intron 16 of TNNT2, Intron 1, Intron 2 and Exon 7 of TNNT3, Intron 5 of TPM1, Intron 1 of ACTC, Exon 1 of MYL3 genes in both HCM and DCM, though the underlying pathophysiology is known to vary / differ. This can be explained on the basis of impaired energy compromise, or dose effect of the mutant protein, or environmental factors wherein an HCM disease phenotype could progress to a DCM phenotype affecting both right and left ventricles, leading to heart failure. Genotype-phenotype correlations can be best explained by phenotypic plasticity where in mutations/ variations in the same gene and even the same mutation/ variation in a different background cause disparate phenotypes, which will be discussed further.

P153

Co-Exposure to Chloroform, Dichloromethane and Toluene Induced Oxidative Stress and Xenobiotic Metabolism Changes but Not Genotoxic Damage. Belmont JA[1], Lopez AP[1], Molina E[1], Serrano L[1], Coballase E[1,2], Cardenas N[1,2], Medina P[1], Montero R[1]. [1]Instituto de Investigaciones Biomedicas, UNAM, DF, Mexico, [2]Laboratorio de neuroquímica. Torre de investigación, INP, DF, Mexico.

Introduction: The mixture of volatile organic compounds (VOCs) chloroform (CLF), dichloromethane (DCM) and toluene (TOL) are the main pollutants described in two rivers of México (Montero2006). These compounds are ubiquitous pollutants in urban environments, but the levels reached in the air due to the pollution in the rivers could be higher, making important the study of this mixture. Objective: The purpose of this study is to investigate the xenobiotic metabolism response, the oxidative stress and the genotoxic damage induced by the co-exposure of CLF, DCM and TOL in order to propose useful biomarkers for human monitoring. Methods: Three groups of rats (8 animals/group) were treated i.p. with different doses of a mixture of CLF, DCM and TOL, a single dose per day, over 3 days. A battery of biomarkers was applied to assess genotoxic damage (HEPMN and MNPCE) oxidative stress (Antioxidant defense and TBARS) and xenobiotic metabolism response (CYP450, CYP2E1, GSTs and GSTT1). Results: Co-exposure to CLF, DCM and TOL decreased antioxidant defenses, altered glutathione redox status (GSH/GSSG) and increased lipid peroxidation. The activity of phase I (CYP450, CYP2E1) and phase II (GSTT-1) enzymes were also modified by the mixture of VOCs and these changes were related to GSH/GSSG ratio. Finally the micronucleus test in bone marrow and hepatocytes did not show a genotoxic effect. In summary, the mixture increased hepatic oxidative stress and changed the activity of phase I and II metabolism enzymes but these toxic effects were not enough to promote the formation of micronuclei.

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Withdrawn.

P155

Chloroquine Autophagy Inhibition Leads to ROS-dependent Cell Killing in Human Glioma Cells. Menck CFM[1], Vessoni AT[1], Vieira DB[1], Cabral-Neto JB[2]. [1]Department of Microbiology, Institute of Biomedical Science, University of São Paulo, São Paulo, SP, Brazil, [2]Instituto de Biofísica Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

Glioblastoma multiform is one of the most common and lethal intracranial tumors, with median survival time of patients of only 12-15 months, even after chemo and/or radiotherapy. Chloroquine (CQ) is a drug widely used for the treatment and prevention of malaria and, recently, it has also been used for the treatment of glioma, complementing radio and chemotherapy, with exciting results. In fact, glioma cells were shown *in vitro* to be sensitive to CQ treatment, depending on the p53 status, wild type p53 being the most sensitive. CQ is known to be a potent inhibitor of autophagy, a survival pathway that allows cells to cope with different stressful situations, such as starvation and DNA damage. CQ-mediated killing can be abolished in the presence of NAC, a scavenger for Reactive Oxygen Species (ROS), suggesting participation of ROS in the toxicity induced by CQ. The ROS production and cell killing by CQ clearly correlate with LC3II accumulation in the cells. LC3II is an important marker of autophagosome formation, and apparently cells enter in an oxidative stress status, which is the main cause of cell CQ-induced cell death. The results also indicate that autophagy inhibition by CQ may lead to increased cell oxidative stress, and this process is a critical mediator of glioma cell killing by this drug. Further experiments are exploiting CQ action in combination with drugs commonly used for glioma treatment. Financial support: FAPESP (São Paulo, Brazil) and CNPq (Brasilia, Brazil).

P156

Evidence for Mutagenicity of 3-Hydroxyanthranilic Acid but Not Anthranilic Acid in the Presence of a Metal Cofactor Cu^{2+} *In Vitro*. Gadupudi GS, Chung K-T. The University of Memphis, Memphis, TN, United States.

Tryptophan metabolites have been implicated in the etiology of cancer, especially bladder cancer. We compared the genotoxicity of tryptophan metabolites anthranilic acid (AA) and 3-hydroxyanthranilic acid (3-OHAA) for their ability to induce mutagenesis *in vitro*. The mutagenicity of tryptophan metabolites was analyzed using plasmid relaxation assay performed with AA and 3-OHAA at varying concentrations between 50 μM and 400 μM in the presence of plasmid pSP-72. Neither AA nor 3-OHAA showed any plasmid relaxation activity when tested alone. However, 3-OHAA could induce plasmid relaxation by causing a nick in the presence of a metal cofactor Cu^{2+} but not with other metal cofactors Fe^{2+} and Mn^{2+}. Cu^{2+} at increasing concentrations of 5-20μM in the presence of 100μM 3-OHAA showed an apparent dose-response in causing DNA strand breaks. The Cu^{2+} mediated mutagenic activation of 3-OHAA was further investigated using Ames *Salmonella*/microsome mutagenicity assay with reactive oxygen species (ROS) sensitive tester strain *Salmonella* TA102. A significant increase in TA102 revertants was observed with an increase in concentration of Cu^{2+} from 2.5-50 μg incubated along with 100 μg 3-OHAA/plate. These results suggest the involvement of reactive oxygen radicals in causing mutagenesis. In contrast, AA was unable to cause any significant increase in TA102 revertants when incubated with Cu^{2+}. This evidence for mutagenicity with only 3-OHAA and Cu^{2+}, but not AA,suggests that the presence of the hydroxyl group in 3-OHAA is critical in reacting with Cu^{2+} to generate ROS. Further research is warranted to characterize the role of 3-OHAA in carcinogenesis.

P157

The Role of Glyceraldehyde-3-Phosphate Dehydrogenase in Mono-Halogenated Acetic Acid Mediated Toxicity. Pals JA, Ang JK, Wagner ED, Plewa MJ. University of Illinois, Urbana-Champaign, IL, United States.

The halogenated acetic acids are a major class of drinking water disinfection byproducts (DBPs) with 5 haloacetic acids regulated by the U.S. EPA. These agents are cytotoxic, genotoxic, mutagenic, and teratogenic. The decreasing toxicity rank order of the monohalogenated acetic acids (monoHAAs) is iodo- > bromo- >> chloroacetic acid. We present data that the monoHAAs inhibit glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity in a concentration dependent manner with the same rank order as above. The rate of inhibition of GAPDH and the toxic potency of the monoHAAs are highly correlated with their alkylating potential and the propensity of the halogen leaving group. This strong association between GAPDH inhibition and the monoHAA toxic potency supports a comprehensive mechanism for the adverse biological effects by this widely occurring class of regulated DBPs.

P158

DNA Damage Profiles Induced by Sunlight at Different Latitudes. Schuch AP[1], Yagura T[1], Schuch NJ[2], Makita K[3], Yamamoto H[4], Agnez-Lima LF[5], Monreal R[6], Menck CFM[1]. [1]University of São Paulo, São Paulo, SP, Brazil, [2]Southern Regional Space Research Center, Santa Maria, RS, Brazil, [3]Takushoku University, Tokyo, Japan, [4]Rikkyo University, Tokyo, Japan, [5]Federal University of Rio Grande do Norte, Natal, RN, Brazil, [6]University of Magallanes, Punta Arenas, Chile.

Despite the increase in knowledge of the biological effects of solar ultraviolet (UV) radiation on human health and ecosystems, the impacts of the combination of its increasing incidence with other factors of global warming and industrial activities are not yet known and difficult to predict. Hence, the development and application of DNA-based biological sensors to monitor the solar UV radiation under different environmental conditions is of increasing importance. With the purpose to demonstrate a molecular perspective of the genotoxic impact of sunlight, field experiments were carried out with the DNA-dosimeter system in parallel to physical photometry of solar UVB/UVA radiation at different latitudes in South America. The evaluation of the DNA damage profiles induced sunlight was achieved by biochemical and immunological approaches based on specific DNA repair enzymes and antibodies. The results clearly show that the genotoxic potential of sunlight varies according to the latitudes indeed, indicating that the induction of oxidized DNA bases are directly dependent on the increase of latitude while the generation of 6-4PP is inversely dependent, the latter being a bio-molecular marker of UVB incidence. This molecular DNA pattern greatly reflects the relative incidence of UVA and UVB energies reaching the ground in a specific latitude. Therefore, this work demonstrates the applicability of this DNA-based biosensor for further and continuous field experiments aimed to register the variations of the genotoxic impact of solar UV radiation.

P159

Digital Detection and Quantification of mtDNA Deletions. Taylor SD[1], Kulawiec M[1,2], Ericson NG[1], Bielas JH[1]. [1]Fred Hutchinson Cancer Research Center, Seattle, WA, United States, [2]University of Washington, Seattle, WA, United States.

Deletions in mitochondrial DNA (mtDNA) are associated with cancer and have been shown to be a driving force behind the premature aging phenotype in mitochondrial mutator mice. Environmental mutagens may increase the rate of mtDNA deletion and thus contribute to disease. As such, it becomes important to delineate the relationships between mutagenic exposure, mtDNA mutagenesis, and disease. To this end, we have developed an ultrasensitive assay, termed the Digital Deletion Detection (3D) assay, to more accurately detect and characterize mtDNA deletions. Using Droplet Digital PCR™ (ddPCR™) technology, enzyme digested mitochondrial genomes are partitioned and individually interrogated within thousands of droplets using TaqMan® probes. The fluorescence of each droplet is subsequently measured allowing for a precise, digital readout of each truncated molecule in the sample population. Amplicons are recovered and sequenced, and deletion breakpoints are determined. Thus, in a single 96-well plate, millions of mitochondrial genomes can be efficiently interrogated with a detection sensitivity of <1 mutant deletion molecule per 10^7 wild type molecules. Using the 3D assay, we characterized induced mtDNA deletions in cells expressing a proofreading deficient mitochondrial polymerase gamma (POLG). We demonstrated a tenfold increase in the deletion frequency after 3 weeks of POLG mutator expression with no apparent change in breakpoint spectrum. Digital Deletion Detection provides an important tool for studying the pathogenesis of mtDNA deletions, the impact of environmental mutagenic agents on mtDNA mutation accumulation, and the development of mtDNA deletions for use as biomarkers for disease.

P160

Cell Type and 3-D Context Prominently Influence Response to HZE Radiation. Sridhara DM[1], Nakagawa H[2], Cucinotta FA[3], Pluth JM[1]. [1]Lawrence Berkeley National Laboratory, Berkeley, CA, United States, [2]University of Pennsylvania, Philadelphia, PA, United States, [3]NASA, Lyndon B. Johnson Space Center, Houston, TX, United States.

High charge and high-energy (HZE) particles are a unique component of the space radiation environment and causes the formation of complex clustered lesions, which are potentially mutagenic. The damage caused by these heavy energetic nuclei is a combination of dense ionization along the particle track and sparsely deposited δ-rays at a distance from the track core. The cellular response to HZE exposure involves the activation of protein phosphorylation cascades. Persistent phosphorylation of certain signaling proteins have been associated with prolonged oxidative stress and increased cancer risk. The signaling of one such protein, ATF2 has been implicated in cancer development. We have hypothesized that the phosphorylation kinetics of ATF2 is context driven and relates to the complexity of radiation damage. Studies were carried out using conventional 2-D tissue culture and an innovative 3-D organotypic culture system, which reconstitutes the *in vivo* physiological context of the esophageal epithelium, to differentiate direct track effects from the δ-ray exposures. Phosphorylation profiles of various repair proteins (gH2aX, pATF2) were examined using novel flow-based assays and immunofluorescence microscopy. Our studies indicate that the phosphosphorylation profile of ATF2 is cell-type dependent. Delayed signaling is indicative of the increased complexity of damage elicited by various radiation qualities. Understanding the contribution of the components of HZE exposure to the biological effectiveness in the tissue context is a question of great relevance to NASA for estimating cancer risk in manned deep space missions and to the field of radiotherapy with the increasing use of heavy ions in cancer treatment.

P161

PCB-Induced Telomerase Reactivation Reversed Telomere Shortening by Increased c-MYC, hTERT, and hTR Expression: A Mechanistic Pathway of PCB Carcinogenesis? Perumal Kuppusamy S[1], Klingelhutz A[2], Robertson L[1], Ludewig G[1]. [1]Interdisciplinary Graduate Program in Human Toxicology, University of Iowa, Iowa City, IA, United States, [2]Department of Microbiology, Iowa City, IA, United States.

Activation of telomerase activity and lengthening of telomeres are key steps in carcinogenesis. Polychlorinated Biphenyls (PCBs), a group of 209 different congeners, are classified as probable human carcinogens. To explore if PCBs affect telomerase and telomeres, immortal human skin keratinocytes were exposed to PCB congeners 28, 126 and 153 at 5μM concentration for 48 days. Cells were re-seeded every 6th day with fresh medium and telomerase activity, telomere length (qPCR), cMyc, hTERT, hTR, CYP1A1 mRNA (RT-PCR), CYP1A1 activity (EROD production), cell cycle distribution (flow cytometry), and superoxide level (DHE oxidation) were determined. All PCB congeners reduced telomerase activity and telomere length. PCB126 caused the most prominent reduction of telomerase activity (50%), hTR and hTERT mRNA (10%), telomere length (40%) and cell growth, along with an increase in CYP1A1 mRNA and activity, and in superoxide levels from day 6 to 48; Treatment with PCB126 was continued until day 90. From day 54 on, an increase in cell growth, cMyc, hTERT, and hTR mRNA level (to 130%) along with re-activation of telomerase activity (to 100%) and re-elongation of telomere length (to 90%) was observed. This increase in cMYC, hTERT, and hTR transcripts after critical telomere shortening may be an indication of genomic instability, a hallmark of carcinogenesis. This study shows for the first time that PCBs initially reduce telomerase activity, telomere length, and cell growth, but prolonged exposure may later lead to telomerase re-activation, telomere lengthening and increased cell growth, all key components in cancer initiation and progression. (Supported by NIEHS P42ES013661.)

P162

Cellular Stress Responses Elicited by Engineered Nanomaterials. Prasad RY[1], Wallace K[2], Blackman CF[2], DeMarini DM[2], Simmons SO[2]. [1]Student Services Contractor, U.S. EPA, Research Triangle Park, NC, United States, [2]Integrated Systems Toxicology Division, U.S. EPA, Research Triangle Park, NC, United States.

Engineered nanomaterials are being incorporated continuously into consumer products, resulting in increased human exposures. The study of engineered nanomaterials has focused largely on oxidative stress and inflammation endpoints without further investigation of underlying pathways. Here we examine biological response pathways affected by engineered nanomaterials using a battery of stable luciferase-reporter HepG2 cell lines. We measured the activation of three key stress-responsive transcription factors (NFκB, NRF2, and AP-1) and the human IL-8 promoter after exposure to 6 titanium dioxide (nano-TiO2) and 2 cerium oxide nanomaterials (nano-CeO2). Exposure concentrations ranged from 1-100 µg/ml per nanomaterial over a time course of 6, 16 and 24 h. Cytotoxicity was measured in parallel using the MTT assay at 24 h. Dynamic light scattering was used to determine the size and zeta potential. There were significant changes in transcriptional activation at concentrations as low as 1 µg/ml. The 10 nm anatase nano-TiO2 elicited the highest effect, a ~2-fold increase in NFkB transcriptional activation, at a concentration of 100 µg/ml after 24 h exposure. NRF2 transcriptional activity was stimulated by one nano-CeO2 (~1.5-fold activation) at 100 µg/ml after 24 h. 1 µg/ml anatase/rutile nano-TiO2 elicited a 1.3-fold increase in AP-1 activity after 24 h. IL-8 activity mirrored NFkB transcriptional activation signature at longer exposures. Both anatase/rutile nano-TiO2 were cytotoxic at 100 µg/ml after 24 h. Our results demonstrate the potential for engineered nanomaterials to elicit cellular stimulation through the NFκB pathway and other stress response pathways. [Abstract does not necessarily reflect the policies of the U.S. EPA.]

P163

Genotoxicity, Replication Dynamics, and Intra-S Checkpoint Signaling in Human Dermal Fibroblasts Treated with Titanium Dioxide Nanoparticles. Prasad RY, Chastain PD, Feinberg NN, Smeester LM, Kaufmann WK, Fry RC. University of North Carolina at Chapel Hill, Chapel Hill, NC, United States.

The growing usage of nanoparticles in consumer products increases their prevalence in the environment and potential risk to human health. Titanium dioxide (nano-TiO2) nanoparticles, for example, are used in sunscreens due to their high refractive index and absorptive properties. Although recent studies have shown *in vivo* and *in vitro* toxicity of nano-TiO2 such as pregnancy complications, inflammation, and genotoxicity, a more detailed understanding of the mechanism by which this occurs is unknown. Here, we report that low concentrations of nano-TiO2 cause DNA damage and replication fork stress in human skin fibroblasts *in vitro*. Using high-throughput chamber slides and immunohistochemistry, we were able to identify significantly greater phosphorylated-H2AX foci at concentrations of 1 and 3 µg/ml after 24 h exposure. Additionally, we found inhibition of the overall rate of DNA synthesis and frequency of replicon initiation events in combed DNA fibers after 1 h exposure that suggests intra-S checkpoint regulation in nano-TiO2 treated fibroblasts. We are investigating the ATM- and ATR-regulated DNA repair pathways to determine if the DNA damage and replication fork stress induced by nano-TiO2 treated fibroblasts mimics the cellular response of known genotoxicants such as UV and/or ionizing radiation. These findings will provide a mechanistic overview of the effects of nano-TiO2 exposure, and potentially offer a paradigm for the cellular response to DNA damage by nanoparticles.

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Silica Nanoparticles Administered at the Maximum Tolerated Dose Induce Oxidative Stress Response Pathway Regulation, Indirectly Causing Genotoxicity. Crosby ME, Downs TR, Pfuhler S. The Procter and Gamble Company, Cincinnati, OH, United States.

The mechanisms of direct *versus* indirect DNA damage may lead to differential threshold models that may be used in risk assessment. Results from our Comet / micronucleus combination assay in which rats were intravenously exposed to small (15 nm) and large (55 nm) silica nanoparticles (NPs) indicate that silica NPs, at a maximal tolerated dose (MTD), can induce genotoxicity. However, these data do not provide sufficient information regarding mode of action (MoA). We hypothesized that silica NPs may initiate inflammation, trigger oxidative stress, and induce antioxidant pathway regulation. Due to the instability of ROS, measuring such species *in vivo* is not practical. Hence, we examined surrogate markers of inflammation and oxidative stress, including IL-6 and TNF-a plasma levels and urinary output of 8-oxodG, an oxidative stress-associated DNA lesion. We measured the redox status of hepatic glutathione and the transcriptional induction of antioxidant pathway-related genes: *Ho-1*, *Gclc, Ogg1,* and *Nr2f2*. Our data indicate that silica NP treatment results in higher TNF-a and IL-6 plasma levels, as compared to control. The smaller silica NPs elicit a slight reduction in the GSH/GSSG ratio, as opposed to the larger silica NPs. The levels of both *Ho-1* and *Gclc* are up-regulated in the small, as compared to the large silica NP-exposed animals. In contrast, both *Nr2f2* and *Ogg1* are down-regulated. There was no detectable change in 8-oxodG. Relative to the large, the smaller NPs potentiated the antioxidant system, suggesting that the genotoxicity detected at the MTD may be caused through indirect DNA damage.

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Carbon Black Nanoparticle Instillation Induces Sustained Inflammation and Oxidative Stress-Related Genotoxicity in Mouse Lung and Liver. Bourdon J[4], Saber A[1], Jacobsen N[1], Jensen K[1], Wallin H[2], Moller P[1], Loft S[2], Yauk C[3], Vogel U[1]. [1]National Research Centre for the Working Environment, Copenhagen, Denmark, [2]Univeristy of Copenhagen, Copenhagen, Denmark, [3]Health Canada, Ottawa, ON, Canada, [4]Univerisity of Ottawa, Ottawa, ON, Canada.

Occupational exposure to carbon black nanoparticles (CBNPs) raises concerns over their safety. CBNPs are mutagenic *in vitro* but less is known about their genotoxicity in various organs *in vivo*. We investigated bronchial alveolar lavage (BAL) cell composition, DNA strand breaks (SBs) and oxidatively damaged DNA in C57BL/6 mice 1, 3 and 28 days post-instillation of 0.018, 0.054 or 0.162 mg Printex 90 CBNPs, alongside controls. The comet assay was used to measure SBs in BAL cells, lung and liver as well as formamidopyrimidine DNA glycosylase sensitive sites (FPG-SS) in lung. BAL cell counts remained elevated for the two highest doses up to 28 days post-exposure ($p<0.001$). Pulmonary SBs occurred at all doses on post-exposure day 1 ($p<0.001$) and remained elevated at the two highest doses until day 28 ($p<0.05$). BAL cell DNA SBs were elevated relative to controls at the highest dose on all post-exposure days ($p<0.05$). Pulmonary FPG-SS was increased at all doses and time-points with significant increases occurring on post-exposure days 1 and 3, in comparison to controls ($p<0.001$-0.05). Hepatic SBs were detected on post-exposure days 1 ($p<0.001$) and 28 ($p<0.001$). BAL cells correlated strongly with pulmonary FPG-SS ($r=0.88$, $p<0.001$), whereas the correlation with SBs were more modest ($r=0.52$, $p=0.08$). The results revealed that deposition of CBNPs in lung induces inflammatory and genotoxic effects in mouse lung that persist considerably after exposure. We also demonstrate that CBNPs cause genotoxicity in both the primary exposed tissue (lung and BAL cells), and in a secondary tissue (liver).

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Building A Novel Nano-Technology Platform: Production and Characterization of Multifunctional Nanolipoprotein Particles. He W[1], Bourguet F[2], Luo J[1], Gao T[1], Huser T[1], Cheng H[1], Henderson P[1], Laurence T[2], Lam K[1], Murphy W[1], Coleman M[1,2]. [1]UC Davis, Davis, CA, United States, [2]Lawrence Livermore National Laboratory, Livermore, CA, United States.

We have developed a novel process of production and assembly of nanolipoprotein particles (NLPs) as reagent for imaging, drug delivery, immunomodulation and stabilizing membrane protein complexes. Nanolipoproteins are components of the human HDL lipoprotein complexes that could be isolated and reconstituted to form NLPs. NLPs are discoidal nanoparticles of 10~20 nm that self-assemble around a phospholipid bilayer. This bilayer mimics closely the cell membrane can support molecules such as dyes and proteins. NLPs present distinct advantages in terms of particle size, monodispersity and consistency: the circular protein belt constrains the dimensions of the bilayer and ensures NLP particle size distributions can be monodispersed and consistent between preparations. NLPs have shown great promise for solubilizing and characterizing membrane proteins and may make many more membrane protein related complexes accessible for biophysical and biochemical study. By the simple addition of a few constituents to cell-free extracts, this approach provides a rapid process for the production of functional soluble membrane protein complexes that eliminates the need for cell growth, cell lysis, and subsequent purification, refolding *etc.* We have demonstrated this process on multiple membrane proteins important for rhodopsin-related proton pumps, drug transporters, host-pathogen interactions, Herceptin receptor family and G-protein coupled receptors. Proteins have ranged in size from 10~200 kDa, with 2 or more transmembrane domains have been shown to be biochemically functional. Our approach represents a unique solution to the challenge of generating soluble and functional polytopic membrane proteins, facilitating the structural and functional characterization of these critical, yet poorly understood molecules.

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Cytotoxic and Genotoxic Effects of Silver Nanoparticles in TK6 Lymphoblastoid Cells. Chen DH[1], Heflich RH[2], Mittelstaedt RA[2]. [1]Little Rock Central High School, Little Rock, AR, United States, [2]National Center for Toxicological Research, Jefferson, AR, United States.

Engineered nanomaterials are being increasingly used as components of consumer products. In particular, silver nanoparticles are widely incorporated into consumer products due to their antiviral, antibacterial, and antifungal effects. However, the toxic risks associated with human exposure to nanomaterials are not completely known. In this study, the micronucleus assay was performed using flow cytometry to assess the cytotoxicity and genotoxicity of 5 nm uncoated silver nanoparticles in TK6 lymphoblastoid cells. Cell cultures were treated in duplicate with 10 - 24 µg/mL of the nanoparticles for 28 hours. Additional cultures were treated with the water vehicle or 0.75 gy of ionizing radiation to serve as the negative and positive controls, respectively. The cytotoxicity of the nanosilver, as measured by relative cell counts, relative increase in cell counts, and relative population doubling, was significantly increased over the negative control in a dose-dependent manner. Cell death rates, measured by apoptosis/necrosis of cells, were increased to 26.4% for the highest dose, compared to 2.4% for the negative control The percentage of micronuclei also was enhanced in a dose-dependent manner. Nanosilver, however, was not considered genotoxic in the *in vitro* micronucleus assay, with micronucleus frequencies in the treated cultures just failing to meet the criterion for a weak positive response at the limit doses set by international guidelines for the assay. The results indicate that 5 nm silver nanoparticles are cytotoxic, but not genotoxic in TK6 lymphoblastoid cells.

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