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In this issue:

Abstracts from the Environmental Mutagenesis and Genomics Society 45th Annual Meeting, September 13–17, 2014, Orlando, Florida

Program Chair: Suzanne M. Morris | New Investigator Co-Chair: Michelle C. DeSimone

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Environmental Mutagenesis and Genomics Society

45th Annual Meeting

Integrating Environmental, Genomic, and Health Research

September 13–17, 2014

Hilton Orlando Lake Buena Vista
Orlando, Florida

Program Chair: Suzanne M. Morris, PhD

New Investigator Co-Chair: Michelle C. DeSimone, PhD

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ANNUAL MEETING AGENDA

FRIDAY, SEPTEMBER 12

3:00 PM–6:00 PM	**REGISTRATION**	**WEST REGISTRATION**
3:00 PM–6:00 PM	**SPEAKER READY ROOM**	**GRAND OFFICE**
7:00 PM–9:30 PM	**EXECUTIVE BOARD MEETING**	**EDELWEISS**

SATURDAY, SEPTEMBER 13

8:00 AM–6:00 PM	**REGISTRATION**	**WEST REGISTRATION**
8:00 AM–6:00 PM	**SPEAKER READY ROOM OPEN**	**GRAND OFFICE**

8:30 AM–11:00 AM **WORKSHOP** **GRAND BALLROOM 6**

Adverse Outcome Pathways
(Separate Registration Required)
Chairpersons: Marilyn J. Aardema, BioReliance Corporation; Darren Kidd, Covance Laboratories Ltd; Kristine L. Witt, National Institute of Environmental Health Sciences; and Carole L. Yauk, Health Canada

8:30 AM	Introduction and Overview of AOPs and the Paradigm Covering Areas Such As What Are Their Uses, Construction, Linkages, and Validation/Identifying Data Gaps *Daniel L. Villeneuve, US Environmental Protection Agency*
9:30 AM	Development, Testing, and Applying AOPs to Risk Assessment *Ian Cotgreave, Karolinska Institute*
9:55 AM	Break
10:10 AM	The AOP Wiki *Stephen W. Edwards, US Environmental Protection Agency*
10:35 AM	AOPs in a Regulatory Framework *Rita Schoeny, US Environmental Protection Agency*

11:00 AM–1:00 PM	**LUNCH ON YOUR OWN**	
11:00 AM–1:00 PM	**COUNCIL MEETING 1**	**HIBISCUS**

1:00 PM–4:00 PM **WORKSHOP** **GRAND BALLROOM 6**

Adverse Outcome Pathways *(continued)*
(Separate Registration Required)
Chairpersons: Marilyn J. Aardema, BioReliance Corporation; Darren Kidd, Covance Laboratories Ltd; Kristine L. Witt, National Institute of Environmental Health Sciences; and Carole L. Yauk, Health Canada

1:00 PM	**Breakout Group 1—Grand Ballroom 6** Mutagenic Mode-of-Action in Cancer *Rita Schoeny, US Environmental Protection Agency*
1:00 PM	**Breakout Group 2—Dogwood** Brainstorming How to Incorporate Epigenetics into AOPs *Catherine B. Klein, New York University School of Medicine*

	1:00 PM		**Breakout Group 3—Camellia** Heritable Effects Mediated through Germ Cell DNA and Chromosome Damage *Francesco Marchetti and Carole L. Yauk, Health Canada*
	2:45 PM		Break
	3:00 PM		**Summary and Discussion—Grand Ballroom 6** *Ian Cotgreave, Karolinska Institute*

4:00 PM–5:30 PM **2015 PROGRAM COMMITTEE MEETING** **EDELWEISS**
(First Meeting)

4:00 PM–5:30 PM **EMGS FORUM** **GRAND BALLROOM 4**
The Times They Are a Changin': Navigating a Science Career in the 21st Century
Chairpersons: Michelle C. DeSimone, New York University; James M. Gentile, Hope College; Catherine B. Klein, New York University School of Medicine; Laura J. Niedernhofer, The Scripps Research Institute; and Jeffrey L. Schwartz, University of Washington

4:00 PM		Introduction *Jeffrey L. Schwartz, University of Washington*
4:05 PM	F1	Current and Future Prospective for Jobs in Science *Catherine B. Klein, New York University School of Medicine*
4:25 PM	F2	So, I Am a Scientist and I Want a Job *James M. Gentile, Hope College*
4:45 PM	F3	Importance of Advocating for Sustaining and Expanding America's Research Enterprise *Laura J. Niedernhofer, The Scripps Research Institute*
5:05 PM		Discussion *Michelle C. DeSimone, New York University*

5:30 PM–6:45 PM **PRESIDENT'S WELCOME RECEPTION** **INTERNATIONAL BALLROOM**
Student and New Investigator Poster Viewing, Exhibits Open

7:00 PM–9:30 PM **EMGS ANNIVERSARY SYMPOSIUM** **GRAND BALLROOM 4**
45 Years of Integrating Environmental, Genomic, and Health Research
Chairpersons: Jack B. Bishop, National Institute of Environmental Health Sciences; George R. Douglas, Health Canada , Stephanie L. Smith-Roe, National Institute of Environmental Health Sciences; Hilde E. van Gijssel, Valley City State University; and Jonathan B. Ward Jr., University of Texas Medical Branch

7:00 PM		Introduction *Jonathan B. Ward Jr., University of Texas Medical Branch*
7:05 PM	S1	A Perspective on the Contributions of EMGS to Characterizing the Effect of Exposure to Environmental Mutagens on the Germ Line and the Risk of Inherited Disease to Future Generations Introduction: *Jack B. Bishop, National Institute of Environmental Health Sciences* Senior Speaker: *Andrew J. Wyrobek, Lawrence Berkeley National Laboratory* Speakers: *Patrick Allard, University of California, Los Angeles and Christopher M. Somers, University of Regina*

7:45 PM	S2	A Perspective on the Contributions of EMGS to Understanding the Role of DNA Damage and Repair in Environmental Mutagenesis Introduction: *Stephanie L. Smith-Roe, National Toxicology Program, NIEHS* Senior Speaker: *Leona D. Samson, Massachusetts Institute of Technology* Speakers: *Stephanie L. Smith-Roe, National Toxicology Program, NIEHS and Thomas E. Wilson, University of Michigan*
8:25 PM		Break
8:40 PM	S3	A Perspective on the Contributions of EMGS to the Quantification of Genomic Damage Induced by Exposure to Environmental Mutagens and the Development of Risk Assessment Strategies for Human Health Introduction: *Hilde E. van Gijssel, Valley City State University* Senior Speaker: *Michael D. Waters, Integrated Laboratory Systems, Inc* Speakers: *Paul Fowler, Unilever and Carole L. Yauk, Health Canada*

SUNDAY, SEPTEMBER 14

7:00 AM–5:00 PM	**REGISTRATION**	**WEST REGISTRATION**
7:00 AM–5:00 PM	**SPEAKER READY ROOM OPEN**	**GRAND OFFICE**
7:00 AM–8:30 AM	**SPECIAL INTEREST GROUP MEETINGS** *(Breakfast on Your Own)*	
	***Combined:* Applied Genetic Toxicology and Risk Assessment Special Interest Groups** *Leaders: Julie A. Cox, University of Ottawa; E. Maria Donner, DuPont Haskell Global Centers; David A. Eastmond, University of California, Riverside; Nagu Keshava, US Environmental Protection Agency; and John J. Nicolette, AbbVie Inc.*	**Grand Ballroom 1**
	Heritable Mutation and Disease Special Interest Group *Leaders: Patrick Allard, University of California, Los Angeles; Christopher M. Somers, University of Regina; and Michael D. Waters, Integrated Laboratory Systems, Inc.*	**Grand Ballroom 2**
	Molecular Epidemiology Special Interest Group *Leaders: Karen Huen Northcote, University of California, Berkeley; Miriam C. Poirier, National Cancer Institute; and Ainsley Weston, National Institute for Occupational Safety and Health, CDC*	**Grand Ballroom 3**
8:30 AM–8:45 AM	**WELCOME** *Ofelia A. Olivero, EMGS President;* *Suzanne M. Morris, EMGS Vice President and 2014 Program Chair ;* *and Michelle C. DeSimone, 2014 New Investigator Program Co-Chair*	**GRAND BALLROOM 4**

8:45 AM–9:45 AM **KEYNOTE SPEAKER (K1)** **GRAND BALLROOM 4**

***In Utero* Development and Gamete Vulnerability**

Chairpersons: Suzanne M. Morris and Kristine L. Witt, National Institute of Environmental Health Sciences

Speaker: Donald R. Mattison, Risk Science International

9:45 AM–12:30 PM **PLENARY SYMPOSIUM** **GRAND BALLROOM 4**

Adverse Health Effects Transmitted through the Female Germ Line

Chairpersons: Janet E. Baulch, University of California; Jack B. Bishop, National Institute of Environmental Health Sciences; Catherine B. Klein, New York University School of Medicine; Francesco Marchetti, Health Canada; John J. Mulvihill, University of Oklahoma Health Sciences Center; and Carole L. Yauk, Health Canada

9:45 AM	S4	Evidence in Humans for the Transmission of Adverse Health Effects through the Female Germ Line *John J. Mulvihill, University of Oklahoma Health Sciences Center*
10:15 AM	S5	Models for the Study of Environmental Impacts on Female Gametogenesis *Jonathan Tilly, Northeastern University*
10:45 AM		Break
11:00 AM	S6	Intergenerational Environmentally-Induced Epigenetic Effects Mediated via the Egg *Dana C. Dolinoy, University of Michigan*
11:30 AM	S7	DNA Repair in Early Embryogenesis: More and Less than You Think *Phyllis R. Strauss, Northeastern University*
12:00 Noon	S8	Genome Analyses of Single Human Oocytes for *In Vitro* Fertilization *Xiaoliang Sunney Xie, Harvard University*

9:45 AM–4:00 PM **POSTERS AND EXHIBITS OPEN** **INTERNATIONAL BALLROOM**

12:30 PM–2:00 PM **LUNCH ON YOUR OWN**

12:30 PM–2:00 PM **COMMITTEE MEETINGS**

(Lunch on Your Own)

Alexander Hollaender Outreach Committee **Jasmine**

Membership and Professional Development Committee **Iris**

Special Interest Group Leaders **Hibiscus**

12:30 PM–2:00 PM **STUDENT AND NEW INVESTIGATOR LUNCHEON** **GRAND BALLROOM 1**

(Advance Registration Required)

2:00 PM–4:00 PM **POSTER SESSION 1 AND EXHIBITS** **INTERNATIONAL BALLROOM**

(Odd-Numbered Posters Attended)

4:00 PM–6:30 PM **APPLIED SYMPOSIUM 1** **GRAND BALLROOM 4**

Health Risks and Benefits from Low-Dose and Low Dose-Rate Environmental Exposures

Chairpersons: Veronica R. Bruce, Lovelace Respiratory Research Institute; William F. Morgan, Pacific Northwest National Laboratory; and Kanokporn Noy Rithidech, Stony Brook University

4:00 PM		Introduction
4:15 PM	S9	Variability: The Common Feature of Induced Genomic Instability *Jeffrey L. Schwartz, University of Washington*
4:35 PM	S10	Radiation-Induced Adaptive and Nontargeted Effects: Modulating Factors and Potential Long-Term Health Consequences *Edouard I. Azzam, UMDNJ-Rutgers New Jersey Medical School*
4:50 PM	S11	Molecular Basis for the Lack of Genomic Instability in Bone Marrow Cells of Mice Exposed to Low-Dose Radiation *Kanokporn Noy Rithidech, Stony Brook University*
5:05 PM		Break
5:20 PM	S12	Health Risks and Benefits from Low Dose and Low Dose-Rate Environmental Exposures *Marianne Sowa, Pacific Northwest National Laboratory*
5:40 PM	S13	Radiation-Induced Modification of Chemical Damage and Risk *Antone L. Brooks, Washington State University*
6:00 PM	S14	Interactions of Low-Dose Radiation and the Carcinogen Benzo[a]pyrene in A/J Mice *Veronica R. Bruce, Lovelace Respiratory Research Institute*
6:20 PM		Discussion

4:00 PM–6:30 PM **BASIC SYMPOSIUM 1** **GRAND BALLROOM 6**

'Omics Biomarkers in Human Studies: Expectations, Success, and Pitfalls

Chairpersons: Stefano Bonassi, IRCCS San Raffaele Pisana; Arthur P. Grollman, Stony Brook University; Meagan Myers, National Center for Toxicological Research, US FDA; and Viktoriya Sidorenko, Stony Brook University

4:00 PM		Introduction *Arthur P. Grollman and Viktoriya Sidorenko, Stony Brook University*
4:05 PM	S15	Multi-'Omics Approaches to Environmental Cancer Risk Factors: A Spotlight on Exposure to Aristolochic Acid and Its Biological Consequences *Jiri Zavadil, International Agency for Research on Cancer*
4:35 PM	S16	Application of 'Omics Technologies in Occupational and Environmental Research *Roel Vermeulen, Utrecht University*
5:05 PM		Break
5:20 PM		Introduction *Stefano Bonassi, IRCCS San Raffaele Pisana and Meagan Myers, National Center for Toxicological Research, US FDA*
5:25 PM	S17	Incorporating Biological, Chemical, and Toxicological Knowledge into Predictive Models of Toxicity *Rita Schoeny, US Environmental Protection Agency*

	5:55 PM	S18	From the Exposome to Disease Platforms and Systems Medicine: The Big Picture Is Now Complete *Stefano Bonassi, IRCCS San Raffaele Pisana*
	6:25 PM		Discussion

6:30 PM–9:00 PM ***EMM*** **EDITORIAL BOARD MEETING** **DOGWOOD**

MONDAY, SEPTEMBER 15

7:00 AM–5:00 PM **REGISTRATION** **WEST REGISTRATION**

7:00 AM–5:00 PM **SPEAKER READY ROOM OPEN** **GRAND OFFICE**

7:00 AM–8:30 AM **SPECIAL INTEREST GROUP MEETINGS**
(Breakfast on Your Own)

DNA Repair and Mutagenic Mechanisms Special Interest Group **Grand Ballroom 1**
Leaders: Andrew B. Buermeyer, Oregon State University; Diane C. Cabelof, Wayne State University; and Robert W. Sobol Jr., University of Pittsburgh Cancer Institute

Environmental Genetic Toxicology Special Interest Group **Grand Ballroom 2**
Leaders: Carol D. Swartz, Integrated Laboratory Systems, Inc. and Jeffrey K. Wickliffe, Tulane University

New Technologies Special Interest Group **Grand Ballroom 3**
Leader: Kevin S. Sweder, Syracuse University

8:45 AM–9:45 AM **KEYNOTE SPEAKER (K2)** **GRAND BALLROOM 4**
Maintaining Genome Stability in the Nervous System
Chairpersons: Suzanne M. Morris and David M. Wilson III, National Institute on Aging, NIH

Speaker: Peter McKinnon, St. Jude Children's Research Hospital

9:45 AM–12:30 PM **APPLIED SYMPOSIUM 2** **GRAND BALLROOM 4**
When Is the Standard Genetic Toxicology Battery Informative for Therapeutic Oligonucleotides?
Chairpersons: Kevin S. Sweder, Syracuse University and Husam Younis, ISIS Pharmaceuticals

9:45 AM	S19	An Overview of the Nonclinical Development of Oligonucleotide-Based Therapeutics *Husam Younis, ISIS Pharmaceuticals*
10:15 AM	S20	What's Next for Genetic Toxicological Testing for Oligonucleotide-Based Therapeutics? *Kevin S. Sweder, Syracuse University*
10:45 AM		Break
11:00 AM	S21	Molecular Mechanisms of DNA Damage Associated with Oligonucleotide (OND) Therapeutics *Mick Fellows, AstraZeneca*
11:30 AM	S22	The Assessment of Oligonucleotide Therapeutics for Genotoxic Potential: A Regulatory Perspective *Peter Kasper, Federal Institute for Drugs (BfArM)*
12:00 Noon		Discussion

9:45 AM–12:30 PM **BASIC SYMPOSIUM 2** **GRAND BALLROOM 6**

Environmental Exposures, Endogenous Processes, and Protective Mechanisms in Neurodegenerative Disease

Chairpersons: PJ Brooks, National Institute on Alcohol Abuse and Alcoholism and National Center for Advancing Translational Sciences; David M. Wilson III, National Institute on Aging; and Luoping Zhang, University of California

9:45 AM	S24	Suppression of Somatic Expansion Delays Motor Decline in a Mouse Model of Huntington's Disease *Cynthia T. McMurray, Lawrence Berkeley Laboratory*
10:15 AM	S25	The Role of DNA Damage Resulting from Oxidative Stress and Lipid Peroxidation in Neurodegeneration in Models Deficient for Nucleotide Excision Repair *Laura J. Niedernhofer, The Scripps Research Institute*
10:45 AM		Break
11:00 AM	S26	Mechanisms and Treatments for Radiation- and Chemotherapy-Induced Cognitive Dysfunction *Charles L. Limoli, University of California, Irvine*
11:30 AM	S27	Formaldehyde Exposure and Amyotrophic Lateral Sclerosis *Marc G. Weisskopf, Harvard University*
12:00 Noon		Discussion

9:45 AM–4:00 PM **POSTERS AND EXHIBITS OPEN** **INTERNATIONAL BALLROOM**

12:30 PM–2:00 PM **LUNCH ON YOUR OWN**

12:30 PM–2:00 PM **WOMEN IN EMGS LUNCHEON** **GRAND BALLROOM 1**

(Advance Registration Required)

Leaders: Meagan Myers, National Center for Toxicological Research, US FDA and Kristine L. Witt, National Institute of Environmental Health Sciences, NIH

12:30 PM		Networking and Introduction of Topic
12:50 PM	L1	Protecting Pregnant Women and Their Babies from Seasonal and Pandemic Influenza *Sonja A. Rasmussen, Centers for Disease Control and Prevention*
1:35 PM		Discussion

2:00 PM–4:00 PM **POSTER SESSION 2 AND EXHIBITS** **INTERNATIONAL BALLROOM**

(Even-Numbered Posters Attended)

4:00 PM–6:00 PM **APPLIED PLATFORM SESSION 1** **GRAND BALLROOM 4**

Chairpersons: Vasily N. Dobrovolsky, National Center for Toxicological Research, US FDA and Michael J. Plewa, University of Illinois, Urbana

Presenting author is underlined.

4:00 PM		Introduction
4:15 PM	1	Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) or Pyruvate Dehydrogenase (PDH) As a Target Molecule for the Toxicity Mechanism of Mono, Di, and Trihaloacetic Acids Water Disinfection By-Products (DBPs) *Dad A, Jeong CH, Wagner ED, Plewa MJ. University of Illinois at Urbana Champaign, IL, United States*

4:30 PM	2	Characterization of BaP-Induced lacZ Mutations in the Bone Marrow and Sperm of Muta™ Mouse Males Using Next-Generation Sequencing *Beal MA[1], Gagne R[2], O'Brien JM[2], Yauk CL[2], Marchetti F[2]. [1]Carleton University, Ottawa, ON, Canada, [2]Health Canada, Ottawa, ON, Canada*
4:45 PM	3	p53 May Be Important for Avoidance of Misleading Positives in *In Vitro* Genotoxicity Tests *Chapman KE, Seager AL, Brüsehafer K, Shah UK, Wilde E, Clatworthy ML, Mikhail JM, Johnson GE, Doak SH, Jenkins GJS. Swansea University, Swansea, West Glamorgan, United Kingdom*
5:00 PM	4	Computational Systems Biology Modeling of DNA-Damage Stress Pathways for Assessing Mutation Rates at Low Doses *Clewell RA[1], Pendse S[1], McMullen P[1], Sun B[1], Zhang Q[1], Adeleye Y[2], Carmichael P[2], Andersen ME[1]. [1]The Hamner Institutes for Health Sciences, Research Triangle Park, NC, United States, [2]Unilever, PLC, Sharnbrook, Bedfordshire, United Kingdom*
5:15 PM	5	Connecting Oxidative Damage to Epigenetic Alterations *Ding N[1], DeStefano Shields C[2], Sears CL[2], Baylin SB[2], O'Hagan HM[1]. [1]Indiana University, Bloomington, IN, United States, [2]Johns Hopkins University, Baltimore, MD, United States*
5:30 PM	6	Large Transcription Units Are Hotspots for Copy Number Variants Induced by Replication Stress *Glover TW, Arlt MF, Park SH, Rajendran S, Paulson M, Ljunjman M, Wilson TE. University of Michigan, Ann Arbor, MI, United States*
5:45 PM	7	CD48-Deficient T-lymphocytes from ENU-Treated Rats Have Mutations in the Pig-a Gene *Dobrovolsky VN, Mittelstaedt RA, Pearce MG, Petibone DM, Revollo J. National Center for Toxicological Research, US FDA, Jefferson, AR, United States*

4:00 PM–6:00 PM **BASIC PLATFORM SESSION 1** **GRAND BALLROOM 6**

Chairpersons: Patrick Allard, University of California, Los Angeles and Robert H. Schiestl, UCLA School of Medicine and Public Health

Presenting author is underlined.

4:00 PM		Introduction
4:15 PM	8	A Yeast Bioassay to Uncover Environmental Mediators of Germ Cell Copy Number Mutagenesis *Argueso JL, Chapman M, Stewart G, Maranon D. Colorado State University, Fort Collins, CO, United States*
4:30 PM	9	Tissue-Specific Transcriptomic Analysis Undermines the Utility of Benzo(a)pyrene As a Point of Reference for Carcinogenic Polycyclic Aromatic Hydrocarbons *Labib S[1], Williams A[2], Guo CH[1], Arlt VM[3], Schmeiser HH[4], Leingartner K[2], Yauk CL[2], White PA[2,1], Halappanavar S[2,1]. [1]University of Ottawa, Ottawa, ON, Canada, [2]Health Canada, Ottawa, ON, Canada, [3]King's College London, London, United Kingdom, [4]German Cancer Research Center, Heidelberg, Germany*
4:45 PM	10	Kinetic Study of Chromosomal Double-Strand Breaks with Diverse Break Structures Using High-Resolution Techniques *Liang Z, Nallasivam S, Wilson TE. University of Michigan, Ann Arbor, MI, United States*

5:00 PM	11	Using High-Throughput Assays to Measure Interindividual Differences in DNA Repair Capacity and to Understand Mechanisms of Cancer Therapy Resistance *Nagel ZD, Margulies CM, Chaim IA, McRee SK, Mazzucato P, Ahmad A, Abo RP, Butty VL, Forget AL, Samson LD. Massachusetts Institute of Technology Cambridge, MA, United States*
5:15 PM	12	Aurora B Mediated Novel Function of DNA Double-Strand Break Response Protein 53BP1 *In Mitosis* *Wang H[1,2], Peng B[3], Engler DA[4], Xu X[3], Xu B[5], Mitra S[1], Hegde ML[1,2]. [1]Department of Radiation and Oncology, The Houston Methodist Hospital Research Institute, Houston, TX, United States, [2]Neurological Institute, the Methodist Hospital, Houston, TX, United States, [3]Beijing Key Laboratory of DNA Damage Response, Beijing, Beijing, China, [4]Proteomics Core, The Houston Methodist Hospital Research Institute, Houston, TX, United States, [5]Department of Biochemistry and Molecular Biology, Southern Research Institute, Birmingham, AL, United States*
5:30 PM	13	Uncovering New Genes, Proteins, and Pathways Regulated by the ARTD1/PARG Axis *Fouquerel E, Yu Z, Li J, Sobol RW. University of Pittsburgh, Pittsburgh, PA, United States*
5:45 PM	14	Effect of Intestinal Microbiota on Lymphoma and Longevity in Atm Deficient Mice *Schiestl R, Liu J. University of California, Los Angeles, Los Angeles, CA, United States*

6:00 PM–7:30 PM **EMGS DEBATE** **GRAND BALLROOM 4**

Use of the Micronucleus Assay for Human Biomonitoring: Successes, Concerns, and Solutions

Moderator: David DeMarini, US Environmental Protection Agency

6:00 PM		Introduction *David DeMarini, US Environmental Protection Agency*
6:10 PM	D1	Does the Recommended Lymphocyte Cytokinesis-Block Micronucleus Assay for Human Biomonitoring Actually Detect DNA Damage Induced by Occupational and Environmental Exposure to Genotoxic Chemicals? *Guenter Speit, University of Ulm*
6:35 PM	D2	Critical Questions, Misconceptions, and a Road Map for Improving the Use of the Lymphocyte Cytokinesis-Block Micronucleus Assay for *In Vivo* Biomonitoring of Human Exposure to Genotoxic Chemicals: A HUMN Project Perspective *Michael Fenech, CSIRO Health Science and Nutrition*
7:00 PM		Discussion

6:00 PM–8:00 PM **EXECUTIVE BOARD MEETING** **HIBISCUS**

TUESDAY, SEPTEMBER 16

7:00 AM–5:00 PM **REGISTRATION** **WEST REGISTRATION**

7:00 AM–5:00 PM **SPEAKER READY ROOM OPEN** **GRAND OFFICE**

7:00 AM–8:30 AM **SPECIAL INTEREST GROUP MEETINGS**
(Breakfast on Your Own)

Epigenetics Special Interest Group **Grand Ballroom 1**
Leaders: Daneida Lizarraga; Janice M. Pluth, Lawrence Berkeley National Laboratory; and Caren Weinhouse, University of Michigan

Transgenic and *In Vivo* Mutagenesis Special Interest Group **Grand Ballroom 2**
Leaders: Alexandra S. Long, Health Canada and Nan Mei, National Center for Toxicological Research, US FDA

8:00 AM–10:00 AM **POSTERS AND EXHIBITS OPEN** **INTERNATIONAL BALLROOM**

8:45 AM–9:45 AM **EMGS AWARD LECTURE (L2)** **GRAND BALLROOM 4**
Personal Genomes, Clan Genomics, and the Clinical Implementation of Genome Analysis
Chairpersons: Suzanne M. Morris and Barbara L. Parsons

Lecturer: James R. Lupski, Baylor College of Medicine

9:45 AM–12:30 PM **APPLIED SYMPOSIUM 3** **GRAND BALLROOM 4**
Advances and Use of Transgenic Rodent Mutation Assays in Risk Assessment: Update on Recent Developments and Applications
Chairpersons: Alexandra S. Long, Health Canada and Nan Mei, National Center for Toxicological Research, US FDA

9:45 AM	S28	Historical Development of Transgenic Rodent Mutation Assays and Future Challenges *Iain B. Lambert, Carleton University*
10:15 AM	S29	Advances in the Transgenic Rodent Assay for Germ Cell Mutagenicity Testing *Francesco Marchetti, Health Canada*
10:45 AM		Break
11:00 AM	S30	Development of Transgenic Hairless Albino Mice for Testing UVB-Induced Circadian Clock in the Skin *Mugimane G. Manjanatha, National Center for Toxicological Research, US FDA*
11:30 AM	S31	Accumulation of Spontaneous Point Mutations and Deletions with Aging in *gpt* Delta Transgenic Rodents *Kenichi Masumura, National Institute of Health Science, Japan*
12:00 Noon	S32	Genotoxicity Testing: *In Vitro* Methods Derived from Transgenic Mice *Mirjam Luijten, National Institute for Public Health and the Environment (RIVM)*

9:45 AM–12:30 PM **BASIC SYMPOSIUM 3** **GRAND BALLROOM 6**
Environmental Epigenetics, Epigenotoxic Assays, and Regulation
Chairpersons: Janet E. Baulch, University of California, Dana C. Dolinoy, and Caren Weinhouse, University of Michigan

9:45 AM	S33	Regulation of Chemicals Based on Epigenetic Liability: Are We There Yet? *Jay I. Goodman, Michigan State University*
10:15 AM	S34	Potential Mechanisms for the Transfer of Environmental Exposures to Epigenetic Change *Mitchell Turker, Oregon Health & Science University*

	10:45 AM		Break
	11:00 AM	S35	Epigenetic Assays: Updates, Benefits, and Limitations *Catherine B. Klein, New York University School of Medic*ine
	11:30 AM	S36	Diverse Epigenetic Enzymes Empower Regulated Gene Expression *Trevor K. Archer, National Institute of Environmental Health Sciences*
	12:00 Noon	S37	Relationships between Mercury Exposure, DNA Methylation, and Cardiometabolic Risk Factors *Jackie Goodrich*, *University of Michigan*

12:30 PM–1:30 PM **LUNCH ON YOUR OWN**

12:30 PM–1:30 PM **EDUCATION, STUDENT AND NEW INVESTIGATOR AFFAIRS COMMITTEE** **MAGNOLIA**
(Lunch on Your Own)

1:30 PM–3:30 PM **APPLIED PLATFORM SESSION 2** **GRAND BALLROOM 4**
Chairpersons: Julie A. Cox, University of Ottawa and Javier Revollo, National Center for Toxicological Research, US FDA

Presenting author is underlined.

1:30 PM		Introduction
1:45 PM	15	Identification of Single Nucleotide Polymorphisms and Novel Genetic Anomalies in the Normalized Transcriptomes of TK6, WTK1, and NH32 Cells by Next Generation Sequencing *Revollo J, Petibone D, Morris S, Ning B, Dobrovolsky V. National Center for Toxicological Research, Jefferson, AR, United States*
2:00 PM	16	Characterization of Primary Muta™Mouse Hepatocytes: A Promising New *In Vitro* Tool for Mutagenicity Assessment *Cox JA[1], Zwart EP[2], Luijten M[2], White PA[1,3]. [1]Department of Biology, University of Ottawa, Ottawa, ON, Canada, [2]Laboratory for Health Protection Research, National Institute for Public Health and the Environment, Bilthoven, Utrecht, Netherlands, [3]Mechanistic Studies Division, Environmental Health Science and Research Bureau, HECSB, Health Canada, Ottawa, ON, Canada*
2:15 PM	17	CometChip: Enabling Translation of DNA Damage and Repair Assays *Ge J, Tay JJI, Su Y, Mazzucato P, Chow DN, Fessler J, Weingeist DM, Wood DM, Engelward BP. Massachusetts Institute of Technology, Cambridge, MA, United States*
2:30 PM	18	MicroRNA-mRNA Regulatory Networks Help to Unravel Mechanisms of Response to BaP Exposure *Lizarraga D[1,2], Gaj S[1], Brauers KJ[1], Timmermans L[1], Kleinjans JC[1], van Delft JH[1]. [1]Netherlands Toxicogenomics Centre, Maastricht University, Maastricht, Netherlands, [2]School of Public Health, University of California, Berkeley, Berkeley, CA, United States*
2:45 PM	19	Characterization of microRNAs in Serum: A New Class of Biomarkers for Diagnosis of Parkinson Disease in Mouse Model *Chigurupati S, Raymick J, Paule MG, Sarkar S. National Center for Toxicological Research, US FDA, Jefferson, AR, United States*

3:00 PM 20 Multiplexed, High Information Content DNA-Damage Response Assay Discriminates Clastogens, Aneugens, and Cytotoxicants
Bryce SM, Bemis JC, Carlson KM, Berg AL, Dertinger SD. Litron Laboratories, Rochester, NY, United States

3:15 PM 21 Multi-Endpoint Comparison of Low-Dose Responses to Benzo(*a*)pyrene
Long AS[1], Arlt VM[2], Dertinger SD[3], White PA[1,4]. [1]Department of Biology, Faculty of Graduate and Postdoctoral Studies, University of Ottawa, Ottawa, ON, Canada, [2]Analytical and Environmental Sciences Division, MRC-HPA Centre for Environment and Health, King's College London, London, United Kingdom, [3]Litron Laboratories, Rochester, NY, United States, [4]Mechanistic Studies Division, Environmental Health Science and Research Bureau, Health Canada, Ottawa, ON, Canada

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1:30 PM–3:30 PM **BASIC PLATFORM SESSION 2** **GRAND BALLROOM 6**

Chairpersons: Janet E. Baulch, University of California and Karen Huen Northcote, University of California, Berkeley

Presenting author is underlined.

1:30 PM Introduction

1:45 PM 22 Impact of Repetitive Element Transcriptional Activation in Cocaine Addiction
Wang T[1], Feng J[2], Fargo D[1], Santos J[1], Nestler E[2], Woychik R[1]. [1] National Institute of Environmental Health Sciences, Durham, NC, United States, [2]Mount Sinai Medical School, New York, NY, United States

2:00 PM 23 Topoisomerase IIa Gene Promoter Methylation Is Higher and Correlates with Cumulative Time of Exposure in Workers Exposed to a Volatile Organic Compound Mixture including Benzene
Jiménez-Garza O[1], Baccarelli A[2], Byun H-M[2], Guo L[3], Zhong J[2]. [1]University of Guanajuato, León Campus, Health Sciences Division, León, Guanajuato, Mexico, [2]Harvard School of Public Health, Laboratory of Human Environmental Epigenomics, Boston, MA, United States, [3]Key Laboratory of Pollution Processes and Environmental Criteria, College of Environmental Sciences and Engineering, Nankai University, Tianjin, China

2:15 PM 24 Short- and Long-Term Epigenetic Effects of Exposure to Six Sources of Particulate Matter
Miousse IR, Chalbot MCG, Nzabarushimana E, Kavouras IG, Koturbash I. University of Arkansas for Medical Sciences, Little Rock, AR, United States

2:30 PM 25 Evaluating Genomic Damages and *GSTM1* and *GSTT1* Polymorphisms in Rural Workers Occupationally Exposed to Pesticides: A Case-Control Study in an Agropastoral Brazilian State
Silva DM[1,2], Carvalho WF[2], Melo COA[1,3], Godoy FR[4], Bastos RP[4], Cruz AD[3,4], Franco FC[1], Arruda AA[1]. [1]Universidade Federal de Goiás, Programa de Pós-Graduação em Genética e Biologia Molecular, Goiânia, Brazil, [2]Universidade Federal de Goiás, Programa de Pós-Graduação em Ciências Ambientais, Goiânia, Brazil, [3]Pontifícia Universidade Católica de Goiás, Mestrado em Genética, Goiânia, Brazil, [4]Universidade Federal de Goiás, Programa de Pós-Graduação em Biotecnologia e Biodiversidade, Goiânia, Brazil

2:45 PM 26 Putative Early-Life Epigenetic Biomarkers of Hepatocellular Carcinoma in Mice Perinatally Exposed to Bisphenol A
Weinhouse C, Nahar MS, Anderson OS, Dolinoy DC. University of Michigan School of Public Health, Ann Arbor, MI, United States

3:00 PM 27 PON1 As a Model for Integration of Genetic, Epigenetic, and Expression Data on Candidate Susceptibility Genes
Huen Northcote K, Yousefi P, Thomas R, Eskenazi B, Holland N. University of California, Berkeley, Berkeley, CA, United States

3:15 PM 28 Overlapping and Distinct Effects of Bisphenol A and Its Substitute BPS on Germ Cells
Chen Y, Allard P. Molecular Toxicology Interdepartmental Program, University of California, Los Angeles, Los Angeles, CA, United States

3:45 PM–4:45 PM **EMGS BUSINESS MEETING** **GRAND BALLROOM 4**

4:45 PM–5:45 PM **TOWN HALL—EMGS ENDOWMENT FUND** **GRAND BALLROOM 4**
Endowment Fund Task Force, Ronald D. Snyder, Chairperson

7:00 PM–10:00 PM **EMGS BANQUET** **INTERNATIONAL BALLROOM**

Awards Presented by Ofelia A. Olivero

Alexander Hollaender Award
EMGS Award
EMGS Service Award
EMGS Emerging Scientist Travel Awards
Student and New Investigator Travel Awards

WEDNESDAY, SEPTEMBER 17

7:00 AM–2:00 PM **REGISTRATION** **WEST REGISTRATION**

7:00 AM–2:00 PM **SPEAKER READY ROOM OPEN** **GRAND OFFICE**

8:00 AM–9:00 AM **COMMITTEE MEETINGS**
(Breakfast on Your Own)

Awards and Honors Committee **Hibiscus**

Finance and Resource Committee **Iris**

Public Relations Committee **Jasmine**

Publication Policy Committee **Edelweiss**

9:00 AM–11:45 AM **APPLIED SYMPOSIUM 4** **GRAND BALLROOM 4**
Toxicogenomics in the Assessment of Mutagenicity and Carcinogenicity: Implications for Risk Assessment (Part 1)
Chairpersons: A. Francina Webster, Health Canada; Russell S. Thomas, National Center for Computational Toxicology, US EPA; and Michael D. Waters, Integrated Laboratory Systems, Inc.

9:00 AM S38 Introduction to the Session and a Review of the Literature
Michael D. Waters, Integrated Laboratory Systems, Inc.

9:30 AM S39 Toxicogenomics *In Vitro*: Gene Expression Signatures for Discriminating Genotoxic from Nongenotoxic Mechanisms
Carol L. Yauk, Health Canada

	10:00 AM	S40	A Large-Scale Toxicogenomic Benchmark Dose Analysis and Resource *Scott Auerbach, National Toxicology Program, NIEHS*
	10:30 AM		Break
	10:45 AM	S41	Toxicogenomics and Dose-Response: Applications to Risk Assessment *Russell S. Thomas, National Center for Computational Toxicology, US EPA*
	11:15 AM	S42	Developing Gene Signatures for Developmental Toxicants in Human Embryonic and Induced Pluripotent Stem Cells *Leslie Recio, Integrated Laboratory Systems, Inc.*

9:00 AM–11:45 AM **BASIC SYMPOSIUM 4** **GRAND BALLROOM 6**

Watching DNA Repair: From Single Molecules to Living Cells

Chairpersons: Bennett Van Houten, University of Pittsburgh Cancer Institute and Hong Wang, North Carolina State University

Underlined author indicates presenter.

9:00 AM	S43	Watching Base Excision Repair Glycosylases Scan for Damage *Susan Wallace, University of Vermont*
9:30 AM	S44	Single-Molecule Imaging Reveals DNA-Binding Properties of Cohesin Proteins SA1 and SA2 *Lin J[1], Kaur P[1], Chen H[2], Countryman P[1], Roushan M[1], Flaherty D[3], Brennan E[1], You C[4], Piehler J[4], Riehn R[1], Tao YJ[2], Wang H[1]. [1]Physics Department, North Carolina State University, Raleigh, NC, United States, [2]Department of Biochemistry and Cell Biology, Rice University, Houston, TX, United States, [3]Genetics Department, North Carolina State University, Raleigh, NC, United States, [4]Division of Biophysics, Universität Osnabrück, Osnabrück, Germany*
9:45 AM	S45	Damage and Repair at Telomeres *Patricia L. Opresko, University of Pittsburgh*
10:15 AM	S46	Cdt2-Mediated XPG Degradation Promotes Gap-Filling DNA Synthesis in Nucleotide Excision Repair *Wang Q-E, Han C, Zhao R, Qian J, Sharma N, Wani G, He J, Zhu Q, Wani A. The Ohio State University, Columbus, OH, United States*
10:30 AM		Break
10:45 AM	S47	Watching Nucleotide Excision Repair Proteins: One Molecule at a Time *Bennett Van Houten, University of Pittsburgh Cancer Institute*
11:15 AM	S48	Live Cell Imaging of Chromatin Remodeling at Lesion-Stalled Transcription *Wim Vermeulen, Erasmus University*

11:45 AM–12:45 PM **LUNCH ON YOUR OWN**

11:45 AM–12:45 PM **2015 PROGRAM COMMITTEE MEETING** **EDELWEISS**

(Second Meeting—Lunch on Own)

12:45 PM–3:15 PM **APPLIED SYMPOSIUM 5** **GRAND BALLROOM 4**

Toxicogenomics in the Assessment of Mutagenicity and Carcinogenicity: Implications for Risk Assessment (Part 2)

A. Francina Webster, Health Canada; Russell S. Thomas, National Center for Computational Toxicology, US EPA; and Michael D. Waters, Integrated Laboratory Systems, Inc.

12:45 PM	S49	Toxicogenomic Analysis of Formalin-Fixed Paraffin-Embedded (FFPE) Samples: A Case Study of the Liver Carcinogen Furan *A. Francina Webster, Health Canada*
1:15 PM	S50	Integration of Genomic Biomarkers for Xenobiotics with Positive Findings in Chromosome Damage Assays *Heng-Hong Li, Georgetown University*
1:45 PM		Break
2:00 PM	S51	Bioinformatics Applications Reveal Gene Regulation Complexities in Response to Toxicant Exposures *Pierre R. Bushel, National Institute of Environmental Health Sciences*
2:30 PM	S52	Reading the Toxicogenomic MAP (TXG-MAP): Modular Gene Expression Changes Associated with Pathogenesis *James L. Stevens, Lilly Research Laboratories*
3:00 PM		Discussion

12:45 PM–3:15 PM **BASIC SYMPOSIUM 5** **GRAND BALLROOM 6**

Topoisomerases

Chairpersons: Michael Smeaton, Stanford University and Ronald D. Snyder

12:45 PM	S53	Naturally Occurring Topoisomerase Inhibitors: The Good, the Bad, and the Bioflavonoids *Neil Osheroff, Vanderbilt University School of Medicine*
1:15 PM	S54	Genomic Instability Induced by Eukaryotic Topoisomerase II *John Nitiss, University of Illinois, Rockford*
1:45 PM		Break
2:00 PM	S55	TopoisomeraseIIβ Is Involved in Initiation of Interstrand Cross-Link Repair in Mammals *Michael Smeaton, Stanford University*
2:30 PM	S56	The Role of Topoisomerase Inhibition in Clastogenicity of Nonalerting Molecules *Ronald D. Snyder*
3:00 PM		Discussion

3:30 PM–5:30 PM **COUNCIL MEETING 2** **BEGONIA**

Environmental Mutagenesis and Genomics Society 45th Annual Meeting Abstracts

(Presenter designated by underlined author.)

Keynote Speaker Abstracts

K1

***In Utero* Vulnerability and Gamete Development.** Mattison DR. Risk Sciences International and University of Ottawa, Ottawa, ON, Canada.

During development, as the gonads and gametes are forming, alterations can be produced in gamete number, structure, and function by intrinsic or extrinsic factors. Over the past decade, molecular and cellular techniques have expanded our understanding of the mode of action of these factors, which influence the number, structure and function of the gametes across the reproductive age. This presentation will summarize those methods and insights they provide concerning *in utero* vulnerability.

K2

Maintaining Genome Stability in the Nervous System. McKinnon PJ. St. Jude Children's Research Hospital, Memphis, TN, United States.

Genome stability is a prerequisite for the development and function of the nervous system. Multiple DNA damage response pathways ensure that DNA lesions resulting from replication stress and other types of damage such as oxidative damage do not impact neural homeostasis. The DNA damage response is especially critical during early neurogenesis when rapid proliferation and progenitor expansion and differentiation generates cellular diversity in the nervous system. For example, numerous congenital human neurologic syndromes are associated with defective DNA damage signaling and compromised genome integrity. These syndromes arise from inactivation of key DNA damage response factors, and can involve diverse neuropathology, including neurodegeneration, neurodevelopmental defects and brain tumors, highlighting the varied tissue-specific needs for neural genome stability. Data from these syndromes and from genetically engineered mouse models have been critical for understanding the physiologic context for different DNA repair pathways. Collectively, these studies have greatly expanded our knowledge of the neural response to genotoxic stress. Thus, understanding how DNA damage signaling pathways promote neural development and preserve homeostasis is essential for understanding fundamental brain function.

Lecture Abstract

L1

Protecting Pregnant Women and Their Babies from Seasonal and Pandemic Influenza. Rasmussen SA. Centers for Disease Control and Prevention, Atlanta, GA, United States.

Pregnant women are at increased risk for influenza-associated complications and death, based on data from previous pandemics and seasonal influenza. This increased risk is related to changes in women's immune, cardiovascular, respiratory, and other systems that occur during pregnancy. Infants, especially those less than six months of age, who are unable to receive the influenza vaccine, are also at increased risk of influenza-associated morbidity and mortality. However, before the 2009 H1N1 pandemic, information on ways to protect pregnant women and their infants from seasonal and pandemic influenza was limited. For example, data on the risks and benefits of treatment with antiviral medications were sparse. Further, influenza vaccination coverage before the pandemic was low, despite the knowledge that pregnant women and their infants less than six months of age were known to be less likely to become ill from influenza if mothers received influenza vaccine during pregnancy. This presentation will summarize lessons learned from the 2009 H1N1 pandemic and how these lessons are being applied to decrease the burden of seasonal influenza and future influenza pandemics on pregnant women and their babies.

L2

Personal Genomes, Clan Genomics, and the Clinical Implementation of Genome Analysis. Lupski JR. Baylor College of Medicine, Houston, TX, United States.

Following the "finished" euchromatic, haploid human reference genome sequence, clinical implementation of genomewide assays for genomic disorder associated copy number variants (CNVs) demonstrated the tremendous utility of capturing rare, unique, and often *de novo* genetic variation for establishing a molecular etiological diagnosis. The rapid development of novel, faster, and cheaper "next generation" massively parallel DNA sequencing technologies is making possible the era of personal human genomics. Personal diploid human genome sequences have been generated and have contributed to better understanding of rare variation in the human genome. We have consequently begun to appreciate the vastness of individual genetic variation from single nucleotide (SNV) to structural variants including CNV. Translation of genome-scale variation into medically useful information is, however, in its infancy. The clinical implementation of personal genome information and the application of whole-genome and exome sequencing to identify the rare genetic susceptibility variants contributing to disease suggest adjuvant therapies. Better analysis tools for determining CNV from sequence and a deeper understanding of the biology of our genome and mechanisms for mutagenesis are necessary in order to continue to decipher, interpret, and optimize clinical utility of what the variation in the human genome can teach us. The function of the majority of human genes remains unknown; interpretation of noncoding variation without a "genetic code" to translate the information is a challenge. Personal genome sequencing may eventually become an instrument of common medical practice, an adjuvant to the family history by providing information that assists in the formulation of a differential diagnosis.

EMGS Debate Abstracts

D1

Does the Recommended Lymphocyte Cytokinesis-Block Micronucleus Assay for Human Biomonitoring Actually Detect DNA Damage Induced by Occupational and Environmental Exposure to Genotoxic Chemicals? Speit G. Ulm University, Ulm, Germany.

The cytokinesis-block micronucleus assay (CBMN assay) is one of the most important assays in genetic toxicology and mutation research. Concerns are expressed with regard to its usefulness for detecting genotoxic effects in populations exposed occupationally or environmentally to DNA-damaging chemicals. It is questioned whether the CBMN assay with cultured human lymphocytes is actually sensitive enough to detect such kind of exposure. Based on the principle of the assay and the available data, increased micronucleus (MN) frequencies in binucleated cells (BNC) are mainly due to MN produced *ex vivo* during the cultivation period. Induction of MN *ex vivo* requires the presence of sufficiently increased damage levels in lymphocytes at the time of sampling and the persistence of damage during cultivation. However, the sensitivity of the CBMN assay is limited because damaged cells can be lost and DNA damage induced *in vivo* can be repaired prior to the production of MN in the presence of cytochalasin B. A comparison with the protocol of the *in vitro* CBMN assay used for genotoxicity testing leads to the conclusion that it is highly unlikely that DNA damage induced *in vivo* is the cause for increased MN frequencies in BNC after occupational or environmental exposure to genotoxic chemicals. This critical assessment casts doubt on the reliability of many published positive results and questions their relevance in hazard identification and risk assessment. Therefore, it seems worthwhile to reconsider the use of the CBMN assay as presently conducted for the detection of genotoxic exposure in human biomonitoring.

D2

Critical Questions, Misconceptions, and a Road Map for Improving the Use of the Lymphocyte Cytokinesis-Block Micronucleus Assay for *In Vivo* Biomonitoring of Human Exposure to Genotoxic Chemicals: A HUMN Project Perspective. Kirsch-Volders M[1], Bonassi S[2], Knasmueller S[3], Holland NT[4], Bolognesi C[5], Fenech MF[6]. [1]Vrije Universiteit Brussel, Brussel, Belgium, [2]IRCCS San Raffaele Pisana, Rome, Italy, [3]Medical University Vienna, Vienna, Austria, [4]University of California, Berkeley, Berkeley, CA, United States, [5]National Institute for Cancer Research, Genova, Italy, [6]CSIRO Health Science and Nutrition, Adelaide, Australia.

The lymphocyte cytokinesis-block micronucleus (CBMN) assay has been applied in hundreds of *in vivo* biomonitoring studies of humans exposed to genotoxic chemicals because it allows measurement of both structural and numerical chromosome aberrations. Because of numerous published studies there is now a need to re-evaluate the use of MN and other biomarkers within the lymphocyte CBMN cytome assay (e.g., nucleoplasmic bridges, NPB, and nuclear buds, NBUD) as quantitative indicators of exposure to chemical genotoxins and the genetic hazard this may cause. Several misconceptions as well as knowledge gaps need to be addressed to make further progress in the proper application of this promising technique and enable its full potential to be realized. The HUMN project consortium recommends a three pronged approach to further improve the knowledge base and application of the lymphocyte CBMN cytome assay to measure DNA damage in humans exposed to chemical genotoxins: i) a series of systematic reviews, one for each class of chemical genotoxins, of studies which have investigated the association of *in vivo* exposure in humans with MN, NPB and NBUD in lymphocytes; ii) a comprehensive analysis of the literature to obtain new insights on mechanisms by which different classes of chemicals may induce MN, NPB and NBUD *in vitro* and *in vivo;* and iii) investigation of the potential advantages of using the lymphocyte CBMN cytome assay together with other complementary DNA damage diagnostics to obtain a more complete assessment of the DNA damage profile induced by *in vivo* exposure to chemical genotoxins in humans.

EMGS Forum Abstracts

F1

Current and Future Prospectives for Jobs in Science. Klein CB. New York University School of Medicine, Tuxedo, NY, United States.

In an economic climate in which federal funding of research continues to decline and the costs of doing science have risen dramatically, the competition for grants and jobs, and especially academic positions in science careers is at an all-time high. EMGS members face ongoing challenges on all fronts. This session will focus on current and future job trends in environmental health sciences, with an emphasis on the types of jobs and the level of education that is required for various job descriptions in basic and applied sciences in environmental health, environmental toxicology, environmental exposures risk assessment and other related job descriptions. It will address the diversity of employment arenas—academia, government and private industry—that are relevant to the EMGS membership. This session will open up discussions that will be of interest to EMGS students, postdocs, early career researchers, and sunset career scientists, as well.

F2

So, I Am a Scientist and I Want a Job. Gentile J. Hope College, Holland, MI, United States.

In a land far away, at a time long ago, I whispered those words to myself so many times that my wife considered having me committed. But, here I was, a PhD stamped somewhere on my derriere, a “hot shot” postdoc under my belt, a few publications in the realm of environmental mutagenesis (at least one of which caused a significant amount of fussing and arguing such that I was sure I would never touch a test tube again) and a world of worry about “...what to do...” facing me. Well, I thought about where my “heart was” and not surprisingly found it was “all about my mentors—people with names like McNabb (did undergraduate research with him), Brockman and Richardson (got an MS and Doctorate with them), Magee (a great postdoctoral mentor), and Coffin and Galston (two greats from back in the day-both who fought the issues of the day-one from the pulpit and one from the lab bench.) Why the story? Because there is an educational and research thread that runs throughout those names. So I did research with each (ok, not with Coffin), starting as an undergraduate and running through today. And I decided to be like them - scientists who took teaching very seriously and the mentoring of student research collaborators even more so. And I thus built a career teaching undergraduates, doing research with many of them, and fostering their careers as best I could. And as that wound down, I looked to see what I could do in the K-12 sector. And found a world of work there too. So, that’s my story and happy to share the “how can I do this” (or something comparable) perspective as part of the panel.

F3

Importance of Advocating for Sustaining and Expanding America’s Research Enterprise. Niedernhofer LJ[1,2]. [1]Board of Directors, Federation of American Societies for Experimental Biology, Bethesda, MD, United States, [2]The Scripps Research Institute, Department of Metabolism and Aging, Jupiter, FL, United States.

Science and medical research in the United States funded by NIH, the NSF, and DOD is supported by 0.4% of the federal budget. Furthermore, funding for scientific research falls into the category of “discretionary budget” meaning that the Senate and Congress must approve money to support research every year. In the last decade, the money allocated to research has not increased at all, leading to a 21% decrease in purchasing power and 34% fewer grants. This unpredictability and failure to steadily invest in research causes a loss in future economic growth for the USA, reduced scientific creativity, fewer jobs and brain drain to other countries. What can and should you do about it? In this session, I will discuss what you can do to advocate for biomedical research in America. In addition, I will provide tools that you can use in this effort. Your willingness to help can have a tremendous impact on funding for research and your job prospects. It can also improve your scientific communication skills.

Symposia Abstracts

EMGS Anniversary Symposium—45 Years of Integrating Environmental, Genomic, and Health Research

S1

A Perspective on the Contributions of EMGS to Characterizing the Effect of Exposure to Environmental Mutagens on the Germ Line and the Risk of Inherited Disease to Future Generations. Wyrobek AJ[1], Allard P[2], Somers CM[3]. [1]Lawrence Berkeley National Laboratory, Berkeley CA, United States, [2]University of California, Los Angeles, Los Angeles, CA, United States, [3]University of Regina, Regina, SK, Canada.

Forty-five years ago, the newly-formed EMS faced the challenges of assessing heritable risks in Japanese bomb survivors and predicting mutational risks from the massive numbers of environmental, medical, and occupational agents to which humans were exposed. Our Society has made significant advances toward understanding gene and chromosomal mechanisms of chemical and radiation mutagenicity and identifying windows of mutational sensitivities and resistance during germ-cell development and after fertilization. About 100 chemicals have been characterized for germ-cell mutagenicity primarily in mouse models, showing 1) chemical-specific variations in potencies during male and female germ-cell mitosis, meiosis, postmeiosis, and postfertilization; 2) that genetic background, gender, age, diet, and physiological status can be significant modifiers of heritable risks; 3) that environmental chemicals can induce epigenetic changes in germ cells that are heritable to the offspring, and 4) that paternally transmitted mutations can arise by misrepair during spermatogenesis or by maternal misrepair in the zygote. However, no germ-cell mutagen has yet been confirmed for human offspring, likely due to technological limitations. There is international pressure to minimize use of whole animals through high-throughput assays that incorporate multiple aspects of germ-line function and chromosome segregation while maintaining relevance and validity for heritable risks in animals. Dramatic advances in genome-scanning technologies and improved model organisms may soon enable efficient genome-wide interrogations of mutational responses in offspring of exposed parents. We are entering a revolutionary period of mechanistic discovery of germ cell mutagenicity and predictive modelling of heritable risks for the 10's of thousands of chemicals and mixtures in our ecosystem.

S2

A Perspective on the Role of EMGS in Understanding the Contributions of DNA Damage and Repair to Environmental Mutagenesis. Samson LD[1], Wilson TE[2], Smith-Roe SL[3]. [1]Massachusetts Institute of Technology, Cambridge, MA, United States, [2]University of Michigan, Ann Arbor, MI, United States, [3]National Toxicology Program, NIEHS, Research Triangle Park, NC, United States.

The EMS/EMGS was launched in 1969 in the wake of Rachel Carson's "Silent Spring" and the creation of what would become the National Institute of Environmental Health Sciences but before the creation of the Environmental Protection Agency. At that time, only two DNA repair pathways were recognized and their importance in genome stability was uncertain. Today, more than 400 DNA repair proteins and ~20 distinct repair pathways are known and their essential role in protecting living organisms from damaging environmental exposures is crystal clear. Dr. Samson will describe the pivotal events leading to our understanding of these pathways, highlighting contributions of EMGS members. We have seen DNA repair develop during the Society's history from a twig on the burgeoning tree of molecular genetics to a branch of science inseparable from our understanding of mutagenic mechanisms. Dr. Wilson will consider how new and developing experimental approaches are driving the next wave of insight into the precise functions of these pathways. As extraordinary molecular machines, DNA repair pathways present many puzzles yet to be solved, including the identification of unknown factors and lesions and how these machines are optimized for action in the nucleus. Dr. Smith-Roe will discuss how this molecular understanding is being integrated into higher-order explorations of the role of DNA repair in protecting biological systems from environmental mutagens. These activities too require an ongoing innovation to meet the challenge of understanding DNA repair as one hub in the connected network of cellular, organismal and ecological responses to environmental insults.

S3

A Perspective on the Contributions of EMGS to the Quantification of Genomic Damage Induced by Exposure to Environmental Mutagens and the Development of Risk Assessment Strategies for Human Health. Fowler P[1], Waters MD[2], Yauk CL[3]. [1]Unilever, Bedford, United Kingdom, [2]Integrated Laboratory Systems, Inc., Research Triangle Park, NC, United States, [3]Health Canada, Ottawa, ON, Canada.

Please note the contributing author's initials precede their contribution. (MDW) With the introduction and evolution of the Ames test, the primary focus of the EMGS shifted from mutagenicity per se to the prediction of carcinogenicity. Although dose-response information was often obtained in both *in vitro* and *in vivo* assays, it was recognized that such information did not sufficiently describe low dose kinetics, or predict carcinogenic potency. Studies generally focused on hazard detection rather than helping to inform risk to humans. In response to such shortcomings, there are currently efforts to more fully understand the quantitative dose relationship between mutation and resultant human diseases. (PF) One driver for this changed emphasis is the need to develop human health risk assessment approaches for cosmetic and personal care ingredients, which do not rely on the use of data generated in animals. Accordingly, in order to make pragmatic risk assessments, integrated approaches based on mechanistic pathway data and detailed exposure analysis alongside quantitative genetic toxicology approaches are being developed to better understand low dose regions of exposure, and hence identify regions of acceptable risk. (CLY) The past decade has also seen advances in the application of genomics technologies to better understand the impact of chemicals on the genome. Predictive genomic signatures provide mechanistic context to chemical toxicities, and the means to identify cancer modes of action, significantly improving the cancer prediction of short-term test batteries. Advances in bioinformatics permit genome-wide dose-response modeling for transcriptional changes, demonstrating an excellent correlation between the benchmark doses derived from sub-chronic *in vivo* assays and 2-year cancer outcomes.

Plenary Symposium—Adverse Health Effects Transmitted through the Female Germ Line

S4

Evidence in Humans for the Transmission of Adverse Health Effects through the Female Germ Line. Mulvihill JJ. University of Oklahoma, Department of Pediatrics, Oklahoma City, OK, United States.

Despite expectations, no excess of germ cell mutations, seen as genetic disease and adverse pregnancy outcomes (APOs), has been documented in children of survivors from atomic bombs in Japan nor in offspring of survivors of childhood, adolescent, and young adult cancer. Reasons for studying offspring of cancer survivors are many: exposures include radiation and various classes of chemicals in a wide range of doses, combinations of agents, and duration. The same subjects can be studied for corollary endpoints, such as additional primary malignancies, somatic cell mutations, and infertility. The subjects are in the health care system, so exact documentation of exposure route, timing, and dose is available, along with potentially confounding factors. Three large retrospective case cohorts in the United States, Canada, Denmark, and Finland (The Five Center Study, The Childhood Cancer Survivors Study, and The Genetic Consequences of Cancer Therapy) comprise 30,891 survivors, their 30,302 pregnancies with a total of 654 offspring with genetic disease, not different from rates in the offspring of the survivors' siblings and from population rates. Each study mentioned no differences by gender of the survivor, but a definitive analysis was not presented. A more complete analysis is underway, but the findings seem reassuring that overall the children of cancer survivors are not at high risk of genetic disease apart from known cancer predispositions. Seeking molecular evidence of germ cell mutation could involve total genomic sequencing in offspring versus both parents.

S5

Models for the Study of Environmental Impacts on Female Gametogenesis. Tilly, JT. Department of Biology, Laboratory of Aging and Infertility Research, Northeastern University, Boston, MA, United States.

For decades it was widely believed that mammalian females are incapable of oocyte generation after birth. Accordingly, essentially all efforts to understand how environmental toxicants negatively impact on the female germline have been confined to studies of embryonic primordial germ cells or oocytes. Outcomes of exposure vary depending on the toxicant, although germ cell apoptosis (*Nature Reviews Molecular Cell Biology* 2001 2:838-848) and meiotic aneuploidy (*Cytogenetics Genome Research* 2011 133:254-268) are two of the most prominent. Over the past decade, a growing body of evidence has challenged the premise that oogenesis ceases at birth, thus opening the possibility that a third principal germline target of environmental toxicants exists in mammalian ovaries – female germline or oogonial stem cells (OSCs) (*Nature* 2004 428:145-150; *Nature Cell Biology* 2009 11:631-636; *Nature Medicine* 2012 18:413-421; *Nature Protocols* 2013 8:966-988). Although little is known of the responses of OSCs to toxicant exposure, we have previously mapped the molecular mechanisms by which polycyclic aromatic hydrocarbons (PAHs) cause premature ovarian failure through transcriptional activation of oocyte apoptosis (*Nature Genetics* 2001 28:355-360; *Reproductive Sciences* 2009 16:347-356). We are currently determining if OSCs are also targeted by PAHs. In addition, we have uncovered through gene knockout studies that OSCs utilize estrogen receptor signaling to engage meiotic differentiation. We have thus expanded our efforts with OSCs to include studies of bisphenol-A and diethylhexyl phthalate, which can act through misdirection of estrogen signaling. An overview of this work will be presented. Supported by NIH R37-AG012279, NIH R21-HD072280, Glenn Foundation for Medical Research.

S6

Intergenerational Environmentally-Induced Epigenetic Effects Mediated via the Egg. Dolinoy DC. University of Michigan, Ann Arbor, MI, United States.

Environmental factors, including toxicants, pharmaceuticals, nutrition, behaviour, and stress, can affect both genetic and epigenetic programming, with particular vulnerabilities during developmental reprogramming of the epigenome. Specifically, the epigenome undergoes two waves of global DNA demethylation followed by *de novo* methylation events. In mammals, the mother, G0, hosts the development of the offspring, F1, from zygote stage to birth. During F1 offspring development, a separate lineage of cells, called the primordial germ cells (PGCs), migrate and differentiate into gamete precursor cells that will eventually become the F2 “grand-offspring” generation. By convention, the “first wave” of epigenetic resetting refers to the reprogramming of the epigenome within these PGCs, and the second wave refers to the reprogramming that happens shortly after zygote formation. In females the PGC remain largely unmethylated until maturation in the F1 adult during each estrous cycle. During fertilization, the F2 gametes combine and undergo the second, more complete, wave of demethylation in preparation for establishment of somatic tissue-specific methylation patterns. Thus, any environmental influences on the pregnant G0 female can affect epigenetic patterning and subsequent adult disease susceptibility intergenerationally—in both the F1 and F2. Using illustrative examples from animal models and humans, this presentation will explore the effects of perinatal environmental factors. We will use endocrine disrupting chemicals as representative environmental exposures and consider the moderating effects of diet, stress, and nutrition. Particular attention will be paid to growth-related imprinted genes, which constitute 1-2 percent of mammalian genome and are monoallelicly expressed depending on the parent-of-origin.

S7

DNA Repair in Early Embryogenesis: More and Less than You Think. Strauss PR. Northeastern University, Department of Biology, Boston, MA, United States.

Most DNA repair studies in early embryos have examined repair of double strand(ds) breaks. However, repair of ds breaks is only one of five DNA repair pathways in eukaryotic cells and far from the most prevalent. Indeed, the most prevalent pathway, found in all eukaryotic cells, is single nucleotide base excision repair (BER). BER consists of five steps that recognize, remove, and repair uracil, oxidized bases and many methylated bases. All mammalian cells studied to date have the full complement of BER enzymes, since oxidative DNA damage due to reactive oxygen species(ROS) is ongoing as a low level byproduct of ATP generation through oxidative phosphorylation. ROS damage lipid, protein and nucleic acid. While damaged protein and lipid can be discarded, oxidized DNA must be repaired or important genetic information will be lost. In mice knockout of DNA polymerase ß (PolB) or AP endonuclease 1 (Apex1), both critical enzymes in the pathway, is an embryonic lethal. polb$^{-/-}$ pups die shortly after parturition, while apex1$^{-/-}$ embryos fail to proceed beyond Day 9 of gestation. While polb$^{-/-}$ embryonic fibroblast cell lines have been developed, no apex1$^{-/-}$ cell line has yet been reported. Despite the importance of BER for normal embryonic development, the only studies of BER in early embryos have been done in zebrafish in this lab. This talk will review novel findings of BER in early zebrafish oocytes/embryos and present new work on BER in mouse oocytes/embryos. Supported by funds from the G. Harold and Leila Y. Mathers Fund, Aid for Cancer Research, and Northeastern University.

S8

Genome Analyses of Single Human Oocytes for *In Vitro* Fertilization. Xie XS[1,2], Tang F[2], Qiao J[3]. [1]Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA, United Staes, [2]Biodynamic Optical Imaging Center, College of Life Sciences, Peking University, Beijing, China, [3]Center for Reproductive Medicine, Third Hospital of Peking University, Beijing, China.

Single-cell genome analyses of human oocytes are important for preimplantation genomic screening in in vitro fertilization. However, it has been hampered by the nonuniformity of single-cell whole-genome amplification. We carried out genome analyses of single human oocytes using multiple annealing and looping-based amplification cycle (MALBAC), a uniform whole genome amplification method. By sequencing the first and second polar bodies or one of the many cells in the blastocyst stage, the MALBAC-based preimplantation genomic screening enables accurate and cost-effective selection of fertilized eggs that are free from chromosome abnormality as well as point mutations associated with Mandelian diseases from either parent.

Applied Symposium 1—Health Risks and Benefits from Low-Dose and Low Dose-Rate Environmental Exposures

S9

Variability: The Common Feature of Induced Genomic Instability. Schwartz JL, University of Washington, Seattle, WA, United States.

Genomic instability, defined as elevated frequencies of mutations in the genome of a cell lineage, is considered to be an important process in carcinogenesis and in the development of resistance in tumors to radiation and chemotherapy treatments. Instability can develop spontaneously and it can develop as a consequence of exposure to radiation or different chemical genotoxic agents. However, genomic instability does not appear to be a single well-defined process with a known target and well described transduction processes. Instead, genomic instability represents a family of very different phenomena with many different phenotypes, likely targets, and transmission processes. Genomic instability presents as changes in nucleic acid sequences, copy number variations (CNV), gene amplification, delayed reproductive death, chromatid and chromosome breaks, chromosome rearrangements, or aneuploidy. It can develop directly from DNA damage induction in a targeted manner or can develop long after exposure and in a nontargeted fashion. It is mediated by unstable DNA lesions, epigenetic and chromatin structure alterations, changes in DDR pathway responses, free radical levels, or production of other clastogenic products. What is observed is often cell type and age dependent. In this presentation, I will summarize some of the characteristics of induced genomic instability, compare them to other low dose-induced phenomena, and consider how the ability to characterize induced genomic instability might aid in developing chemopreventive approaches as well as improvements in cancer therapy.

S10

Radiation-Induced Adaptive and Nontargeted Effects: Modulating Factors and Potential Long-Term Health Consequences. Azzam E. Rutgers University, Newark, NJ, United States.

The health risks of low level ionizing radiation are the subject of debate. To reduce the uncertainty in evaluating these risks, research advances in cellular and molecular biology are being used to characterize the biochemical effects of low dose/low fluence exposures and their underlying mechanisms. Radiation type, dose rate, genetic susceptibility, cellular metabolic state, levels of biological organization and environmental parameters are among the factors that modulate interactions among signaling processes that determine the outcome of such exposures. Whereas, recommended radiation protection guidelines assume a linear dose-response relationship in estimating radiation health risks, investigations of phenomena such as adaptive responses and bystander effects suggest that low dose/low fluence-induced signaling events act to alter linearity of the dose-response relation as predicted by the biophysical argument and classic target theory. Using normal human cells maintained in culture and a variety of biological endpoints, we have shown that exposures to low doses of sparsely ionizing radiations such as cesium-137 triggers signaling events that protect cells from endogenous oxidative damage or damage due to a subsequent challenge dose of ionizing radiation. In contrast, when cells were exposed to low fluences of densely ionizing radiations such as alpha particles, oxidative stress induced in the traversed cells spread to nearby bystander cells. The long-term effects were more complex because the oxidative effects persisted in progeny of the irradiated and affected bystander cells. Thorough understanding of these effects in the context of systemic responses will likely reduce the uncertainty in estimating the risk of their health hazards.

S11

Molecular Basis for the Lack of Genomic Instability in Bone Marrow Cells of Mice Exposed to Low-Dose Radiation. Rithidech KN[1], Jangiam W[1], Gordon C[1], Whorton E[2]. [1]Department of Pathology, Stony Brook University, Stony Brook, NY, United States, [2]Institute of Human Infections and Immunity, UTMB, Galveston, TX, United States.

Previously, we found that 0.05 Gy of ^{137}Cs-gamma rays (the existing-limit for radiation-exposure in the workplace) was incapable of inducing *in vivo* genomic instability in bone-marrow cells of exposed-mice, regardless of the levels of the repair enzyme DNA-dependent protein-kinase catalytic-subunit (DNA-PKcs). However, higher doses of radiation (0.1 and 1 Gy) do induce genomic instability in mice with intermediate (BALB/cJ) and extremely low-levels (SCID) of DNA-PKcs activity. We investigated the potential molecular mechanisms involved in the lack of genomic instability in bone-marrow cells after exposure of BALB/cJ mice to low-dose radiation. We gave groups of mice various doses of ^{137}Cs-gamma rays (0, 0.05, 0.1, or 1.0 Gy). Mice exposed to 0 or 1.0 Gy of radiation serve as sham- or positive-control, respectively. We measured the levels of activated nuclear factor-kappa B (NF-kB) and related pro-inflammatory cytokines, coupled with the levels of 5-methyl-cytosine and 5-hydroxymethyl-cytosine, in bone-marrow cells collected at 6 months post-irradiation. We found that 0.05 Gy of radiation is incapable of inducing activated NF-kB or pro-inflammatory cytokines. In contrast, both 0.1 and 1.0 Gy of radiation induced significant increases in the levels of these proteins. Further, 0.1 and 1.0 Gy (but not 0.05 Gy) of radiation induced a significant reduction in the levels of 5-hydroxymethyl-cytosine. These findings suggest the role of chronic-inflammation and aberrant-profiles of 5-hydroxymethyl-cytosine in radiation-induced genomic instability. Importantly, our new set of data show that 0.05 Gy is incapable of inducing chronic-inflammation and aberrant-profiles of 5-hydroxymethyl-cytosine, two biological pathways associated with genomic instability. Research funded by NASA Grant# NNX11AK91G.

S12

Health Risks and Benefits from Low Dose and Low Dose-Rate Environmental Exposures. Sowa MB. Pacific Northwest National Laboratory, Richland, WA, United States.

There is no doubt that exposure to high doses of toxic agents (delivered at high dose-rate) includes deleterious health effects. However, assessment of potential health risks associated with exposure to low dose levels (delivered at a low dose-rate) is still a challenging public health issue. Currently, there is a large body of evidence demonstrating differences between biological responses to low dose/low dose-rate and high dose/high dose-rate. Further, there is a substantial volume of published data that opposes the linear no-threshold (LNT) model for radiation-induced cancers. Nonetheless, the health risks and benefits of exposure to low levels of toxic agents have been intensely debated. Since low-dose radiation is ubiquitous in the environment and its uses in daily life (e.g., medical diagnosis or airport safety) is increasing, the potential health risks and benefits of low-dose vs. high-dose radiation will be the key issue of this symposium.

S13

Radiation-Induced Modification of Chemical Damage and Risk. Brooks AL. Washington State University, Kennewick, WA, United States.

At all levels of biological organization, the response to radiation exposure is very dependent on the radiation dose and dose-rate. Low doses of radiation often produce adaptive protective responses against many chemical carcinogens while high doses can act synergistically to increase the damage and risk from the chemical exposures. For example, when radon exposure is combined with cigarette smoke there is a marked increase in the frequency of lung cancers in humans. In contrast, small doses of radiation can protect against chemically induced lung cancer. In many experimental systems, it has been observed that low doses of radiation modify mutation, cell transformation, and carcinogenic effects of chemicals. This presentation will review these synergistic and adaptive protective modifications at all levels of biological organization from the molecular to the whole animal. The changes in mechanisms of action as a function of dose and dose-rate will be reviewed to help explain these unique interactions of radiation with chemicals in the production of biological changes.

S14

Interactions of Low-Dose Radiation and the Carcinogen Benzo[a]pyrene in A/J Mice. Bruce VR[1,2], Belinsky S[1], Gott K[1], March T[3], Scott B[1], Wilder J[1]. [1]Lovelace Respiratory Research Institute, Albuquerque, NM, United States, [2]University of New Mexico, Albuquerque, NM, United States, [3]Independent Consultant, Albuquerque, NM, United States.

Low-dose radiation (LDR) activates the immune system and may lead to lung cancer suppression. We investigated the effects of low doses of gamma radiation combined with the carcinogen, Benzo[a]pyrene (B[a]P), in A/J mice. Two study designs were implemented: a long term study to determine effects of LDR on B[a]P induced lung tumor burden and short term studies designed to take a snapshot look at the effects of B[a]P and LDR on the immune system. In our long term study, we observed a suppressed progression of B[a]P-induced lung tumors by LDR when mice were given a bolus dose of B[a]P one month prior to biweekly exposures to six fractionated doses of 100 mGy gamma radiation (Bruce V., et al., Dose-Response 2012; 10:516-526). Short term studies revealed B[a]P- and LDR-induced changes in immune cell phenotype in lung and spleen tissue as well as cytokine secretion derived from cultured splenocyte supernatants. These changes were observed up to one week after administration of a single dose of B[a]P or a single exposure to 10 or 100 mGy gamma rays. B[a]P is cytotoxic to immune cells in these tissues however increases neutrophils in the lung. Additionally, B[a]P promotes a pro-inflammatory cytokine response by increasing IL-1beta, IL-6, IL-17 and TNF-alpha. In contrast, LDR is not cytotoxic in either tissues and increases anti-inflammatory cytokines IL-2, IL-4, and IL-10. We are currently investigating cytokine production in the lung following these treatments as well as identifying the cellular sources of these cytokines in both lung and spleen tissues.

Basic Symposium 1—'Omics Biomarkers in Human Studies: Expectations, Success, and Pitfalls

S15

Multi-'Omics Approaches to Environmental Cancer Risk Factors: A Spotlight on Exposure to Aristolochic Acid and Its Biological Consequences. Zavadil J. International Agency for Research on Cancer, Lyon, France.

Various 'omics methodologies are increasingly considered an integral part of molecular epidemiology studies of cancer, mainly for their potential to reliably identify exposure:disease associations in biospecimens from population-based studies and in experimental model systems. However, numerous challenges remain to be addressed, particularly for ill-defined cancer risk factors. We apply 'omics techniques to study exposure to aristolochic acid (AA) and its biological effects. AA is a herbal nephrotoxin and carcinogen (IARC Group 1) that causes severe renal disease and gives rise to urothelial carcinomas marked by a unique somatic mutation signature recently identified by genomic approaches. Due to the widespread use and occurrence of the *Aristolochia* herbs, dietary intake of AA poses significant disease risk for millions of individuals worldwide but it also exemplifies exposure, which can be reduced and potentially eliminated by evidence-based preventive strategies. Using 'omics, we aim to identify biomarkers of AA exposure, the molecular bases of the renal disease and associated cancers, and to identify molecular markers of recurrent disease. The approaches include massively parallel sequencing, transcriptomics (mRNA and miRNA profiling), and proteomics applied to human biospecimens as well as model systems. Importantly, we also employ powerful computational strategies to systematically query public cancer genomics databases for tumors exhibiting the AA exposure signature. Various levels of integration of these approaches into multi-'omics framework will be discussed alongside their applicability to identifying new populations at risk and detecting new AA exposure-associated tumor types. 'Omics-based opportunities for facilitating clinical management of patients with recurrent cancers will also be highlighted.

S16

Application of 'Omics Technologies in Occupational and Environmental Research. Vermeulen R. Institute for Risk Assessment Sciences, Utrecht University, The Netherlands.

There are several contributions that molecular epidemiology can and have made to the study of occupational and environmental disease. These include among others 1) elucidation of the exposure/dose-response relationships; 2) evaluating biological plausibility that an exposure may be related to an adverse outcome; and 3) providing insight in the biological mechanisms involved in the exposure-disease relationship. There are several examples that can be given where indeed molecular data has had an important contribution in the risk evaluation (e.g. ethylene oxide, trichloroethylene, benzene, and formaldehyde). In these examples inferences were made mostly on single biomarkers. With the advance of OMICs technologies the expectation was/is that we would move from these single markers of effect to the utilization of complex sets of biological markers reflecting phenotypical responses (i.e. signatures) in a systems biology approach. This in turn would allow for comparative molecular epidemiology in which on the basis of these signatures one could predict human health effects. Although steps have been made successful examples are still limited possibly due to limitations in study designs, laboratory-analyses, and biostatistics.

S17

Incorporating Biological, Chemical, and Toxicological Knowledge into Predictive Models of Toxicity. Schoeny R, US Environmental Protection Agency, Washington, DC, United States.

EPA released a *Framework for Human Health Risk Assessment to Inform Decision Making,* that stresses “fit-for-purpose” of the assessment; to meet risk management information needs, assessments may range from prioritization for testing to very sophisticated integrated assessments. Complicating EPA’s diverse assessment needs is the resource intensive nature of traditional toxicological studies. To meet these challenges, EPA is collecting data from new technologies, mining novel data sources, and developing computational tools for analysis and interpretation. For example, the Tox21 program is screening ~8500 chemicals using ~40 *in vitro* assays for efficient evaluation of a chemical’s potential to interact with biological targets or to disrupt cellular processes related to adverse human health effects. The results from the high-throughput *in vitro* assays are placed in a dose context using additional *in vitro* pharmacokinetic assays and *in vitro*-to-*in vivo* extrapolation modeling. This allows a human oral equivalent dose (mg/kg/day) to be calculated for each *in vitro* assay. In addition to characterizing hazard and dose, high throughput exposure models are being developed under the ExpoCast program; these predict exposure potential based on key aspects of chemical fate and transport and personal use. Use of information on the chemical’s mode-of-action and/or knowledge of adverse outcome pathways improves linkages between steps in the assessment source to outcome paradigm. A goal of our integrated research effort is rapid, risk-based prioritization that combines chemical screening data and predictive models for both hazard (ToxCast) and exposure (ExpoCast). This abstract does not necessarily reflect US EPA policy.

S18

From the Exposome to Disease Platforms and Systems Medicine: The Big Picture Is Now Complete. Bonassi S, IRCCS San Raffaele Pisana, Rome, Italy.

The concept of the exposome, representing the totality of exposures from gestation onwards, has been introduced as a complement to genomic data in the study of disease etiology. The original purpose of such investigations was the discovery of key biomarkers of exposure, to be used in exploring hypotheses about sources of exposure, dose-response relationships, mechanisms of action, disease causality and public health interventions. On the other hand, the implementation of systems approaches in clinical practice, and the development of complex predictive models, which, under the four P’s perspective, include social, psychological, and life-style parameters in the therapeutic process, has determined the inclusion of epidemiologic knowledge into clinical studies. The possibility to model in the same playground the complexity of the exposome and the variety of real clinical practice will help to identify new ontologies and to improve disease recognition and treatment.

Applied Symposium 2—When Is the Standard Genetic Toxicology Battery Informative for Therapeutic Oligonucleotides?

S19

An Overview of the Nonclinical Development of Oligonucleotide-Based Therapeutics. Younis HS. ISIS Pharmaceuticals Inc., Nonclinical Development, Carlsbad, CA, United States.

Oligonucleotide-based therapeutics (ONTs) have been investigated over the last 20 to 30 years for the treatment of human disease, and their promise as a new drug modality is now being realized. An overview of the chemical and mechanistic classes of ONTs will be the subject of the presentation with emphasis on describing the general strategies used in developing the nonclinical safety program for this relatively new class of molecules. The general characterization of class- and chemistry-dependent toxicity observed in aniaml species used for nonclinical safety assessment will also be discussed. The most well described pharmacologic mechanisms for synthetic ONTs include the antisense targeting of specific RNA transcripts and aptameric targeting of proteins, while numerous others exist (e.g., modulation of pre-mRNA splicing, inhibition of mRNA translation, exon skipping and modulation of non-coding RNA). In general, ONTs within each chemical class share similar physicochemical and pharmacokinetic properties (e.g., length, solubility, charge-to-mass ratio, hydrophilicity, tissue distribution, metabolism, and protein binding, among others). Thus, the toxicologic properties of ONTs are qualitatively similar within a chemical class, as a whole. To date, the preclinical and clinical experience gained with various classes of ONTs suggest that this relatively new therapeutic modality has a sufficient tolerability profile to support safe evaluation in numerous therapeutic indications.

S20

What's Next for Genetic Toxicological Testing for Oligonucleotide-Based Therapeutics? Sweder KS. Syracuse University, Syracuse, NY, United States.

The Oligonucleotide Safety Working Group (OSWG) has made several recommendations for genetic toxicity testing for therapeutic oligonucleotides (ONs). Genetic toxicity testing for ON therapeutics has been consistently negative. So ON therapeutics that contain well characterized chemical modifications (PS, MOE, LNA, cET) are considered to have low potential for genotoxicity and genetic toxicity testing of such ONs is no longer warranted. For ONs that contain novel chemistry or employ components such as conjugates/linkers or delivery systems, genotoxicity testing is recommended due to the hypothetical concern that modified nucleosides could be liberated from the ON and incorporated into DNA leading to chain termination or mutagenesis. The recommended testing battery is similar to Option 1 of ICH S2(R1). For ONs, evidence of uptake into the cell types used for genotoxicity testing should be documented, as this cannot be assumed due to their large size. Thus, testing in mammalian cells is considered more relevant primarily due to greater potential for drug uptake. Beyond the OSWG recommendations, if oligonucleotide therapeutics incorporate new chemical moieties that have not been investigated previously in standard genetic toxicological assays, which technologies should be considered for testing such new oligonucleotide therapeutics? Since most oligonucleotides do not get into bacteria, and prove uninformative regarding mutagenicity, testing in a mammalian cell system should be considered. Typically, the mouse lymphoma *tk*- assay has proven effective for detecting clastogenic and mutagenic changes following exposure to genotoxic substances. However, what newer technologies might be incorporated into genetic toxicology testing to ensure more rapid, reliable assessment of genotoxicity?

S21

Molecular Mechanisms of DNA Damage Associated with Oligonucleotide (OND) Therapeutics. Saleh A[1], Fellows MD[1], Gooderham N[2], Priestley C[1]. [1]AstraZeneca R&D, Alderley Park, United Kingdom, [2]Imperial College, London, United Kingdom.

The studies by Wang et al., 1996 reporting triplex forming oligonucloetides (TFOs) could induce genotoxicity in a SupF reporter construct[1]. However, Reshat et al., 2012 found no evidence of genotoxicity with a TFO-like sequence targeting the genomic hprt locus[2] and we, amongst others, have demonstrated that minor changes to the sequence used by Wang reduced the SupF positive response. Reshat et al. also assessed the genotoxicity of modified OND degradation products and showed that an adenosine phosphorothioate OND (dAMPαS) gave a potent genotoxic response at the tk locus of TK6 cells[3]. Accordingly, the genotoxicity of dAMPαS was assessed in a variety of genotoxicity assays. The genotoxic response of dAMPαS at tk locus of TK6 cells was confirmed. No mutation was observed at the hprt locus of TK6 cells, the tk locus of L5178Y mouse lymphoma cells and there was little increase in TK6 micronuclei. Furthermore, when TFT resistant TK6 colonies from high concentrations of dAMPαS were rechallenged with TFT, their TFT resistance was lost, indicating that dAMPαS was not mutagenic but was interfering with the TFT selection system leading to unstable phenotype. These investigations provide evidence that therapeutic OND sequences that are considered antisense or antisense-like and their modified bases should not be grouped with the same genotoxic risk as TFOs. Moreover, caution should be used when interpreting data from mammalian cell gene mutation assays with modified nucleotides. [1]Wang et al. (1996) *Science*, 271, 802-805; [2]Reshat et al. (2012) *Mutagenesis*, 27, 713-9; [3]Reshat et al. (2012). *Toxicol Sci*, 130, 319-27.

S22

The Assessment of Oligonucleotide Therapeutics for Genotoxic Potential: A Regulatory Perspective. Kasper P. Federal Institute for Drugs and Medical Devices (BfArM), Genetic and Reproductive Toxicology, Bonn, Germany.

Therapeutic oligonucleotides (ONs) are in the scope of the ICH S6 guideline “Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals” (although ONs are usually chemically synthesized). This guideline states that genetic toxicology testing is not needed unless there is a “cause for concern.” While ICH S6 does not specify what might cause a concern for genotoxicity of ONs, a Reflection Paper of the European Medicines Agency (EMA, 2005) provides more clarity in this respect. Two issues are identified by the EMA document that needs to be addressed in the assessment of potential genotoxicity of new ONs: a) degradation products of chemically modified ONs being nucleotide analogues might lead to mispairing and thus point mutation when integrated into newly synthesized DNA; and b) site-specific mutations might be induced by triplex formation of the ONs with the DNA fiber. The EMA reflection paper considers the standard battery as sufficiently appropriate for testing genotoxicity as a result of incorporation of modified nucleobases into DNA. Demonstration of cellular uptake of testing material to support negative findings would be an important part of this assessment. Regarding site-directed mutagenesis in relation to hypothetical triplex formation non-standard testing approaches would be needed according to the EMA paper such as restriction fragment length polymorphism or other PCR-based techniques. This talk will discuss whether the current regulatory documents provide a sufficiently adequate and up-to-date guidance for assessing potential genotoxicity of ONs.

S23

Abstract withdrawn.

Basic Symposium 2—Environmental Exposures, Endogenous Processes, and Protective Mechanisms in Neurodegenerative Disease

S24

Suppression of Somatic Expansion Delays Motor Decline in a Mouse Model of Huntington's Disease. Budworth H[1], Harris F[2], Williams P[1], Lee D-Y[3], Pahnke J[4], Szczesny B[5], Mitra S[6], Acevdeo-Torres K[7], Ayala-Pena S[8], McMurray CT[1]. [1]Life Sciences Div., Lawrence Berkeley Natl. Laboratory, Berkeley, CA, United States, [2]Molecular Pharmacology and Experimental Therapeutics Dept., Mayo Clinic and Foundation, Rochester, MN, United States, [3]Advanced Fermentation Fusion Science and Technology Dept., Kookmin Univ., Seoul, Democratic People's Republic of Korea, [4]Neurology Dept., Neurodegeneration Research Lab, Univ. of Magdeburg and German Ctr. for Neurodegenerative Diseases, Magdeburg, Germany, [5]Anesthesiology Dept., The Univ. of Texas Medical Branch, Galveston, TX, United States, [6]Radiation Oncology, Houston Methodist Research Inst., Houston, TX, United States, [7]Puerto Rico Ctr. for Inherited Diseases, Univ. of Puerto Rico, San Juan, Puerto Rico, [8]Pharmacology and Toxicology Dept., Univ. of Puerto Rico, San Juan, Puerto Rico.

Huntington's Disease (HD) is a devastating condition that is caused by inheritance of a single allele with an expanded CAG repeat. Postmortem brains of HD patients display somatic expansion of CAG tracts, but whether this promotes toxicity has been unclear, since the inherited protein is also expressed. To address this problem, we created a novel mouse model in which toxicity arising from an inherited and somatic expansion can be measured independently in the same genetic background. We previously reported that the 7,8-dihydro-8-oxo-guanine (8-oxo-G) glycosylase (OGG1) is not essential for life, but its role in base excision repair of oxidative DNA damage causes genetic instability at CAG repeats. We created a more physiological model by crossing *HdhQ150(-/+)* "knock-in" mice with *OGG1(+/-)* mice to produce *HdhQ150(+/+)/OGG1(-/-)* and *HdhQ150(+/-)/OGG1(-/-)* progeny that express the expanded full-length mutant HD allele at endogenous levels with a reduced complement or entirely lacking OGG1. We report that loss of somatic expansion in the *HdhQ150(+/+)/OGG1(-/-)* crosses delays the onset of disease by around 8 months relative to their *HdhQ150(+/+)* littermates, although they both inherit a similar disease-length HD allele. The results provide the first evidence that blocking somatic expansion is beneficial, and widens the therapeutic window for treating more than a dozen deadly human diseases. Therapeutic treatment with antioxidants also suppresses expansion and delays motor decline.

S25

The Role of DNA Damage Resulting from Oxidative Stress and Lipid Peroxidation in Neurodegeneration in Models Deficient for Nucleotide Excision Repair. Beck AP[1], Robinson AR[2], Farrell K[2], Wang J[3], Wang Y[3], Niedernhofer LJ[1,2]. [1]The Scripps Research Institute, Department of Metabolism and Aging, Jupiter, FL, United States, [2]University of Pittsburgh Cancer Institute, Pittsburgh, PA, United States, [3]University of California, Riverside, Department of Chemistry, Riverside, CA, United States.

ERCC1-XPF is a nuclease required for nucleotide excision repair (NER) of helix-distorting lesions as well as other DNA repair pathways. Mutations in *XPF* are linked to a spectrum of genome instability disorders including XP, Cockayne syndrome, Fanconi anemia, and a progeroid syndrome. All patients display some extent of neurodegeneration manifested as progressive cognitive impairment, hearing loss, visual impairment, microcephaly, dystrophia and ataxia. *Ercc*$^{1-/\Delta}$ mice, expressing 10% of the normal level of ERCC1-XPF, model this neurodegenerative process. The mice develop symptoms associated with neurodegeneration starting at eight weeks of age, which progress rapidly and includes dystonia, tremors, ataxia, cerebral atrophy, loss of vision and hearing. Deletion of Ercc1 only in the neurons of the forebrain using the CaMKIIα-Cre transgene led to a profound loss of neurons in the central nervous system and dramatic behavioral changes. Thus, the *Ercc*$^{1-/\Delta}$ mice offer a promising model for neurodegeneration caused by defects in NER. We measured cyclopurine adducts, a substrate for NER, in *Ercc*$^{1-/\Delta}$ and Xpa$^{-/-}$ mice. Cyclopurine lesions accumulate in the liver and kidney of *Ercc1*$^{-/\Delta}$ mice as they age. In contrast, in the brain, the adduct levels peak early and decline with age due to a loss of damaged cells. In *Xpa*$^{-/-}$ mice, cyclopurine adducts are significantly elevated compared to normal controls but accumulate more slowly than in the *Ercc*$^{1-/\Delta}$ mice, correlating with their lack of a neurodegenerative phenotype. These data support the conclusion that oxidative DNA lesions typically repaired by NER promote the loss of neurons once adducts reach a certain level.

S26

Mechanisms and Treatments for Radiation- and Chemotherapy-Induced Cognitive Dysfunction. Limoli C, Parihar V, Tran K, Craver B, Chmielewski N, Baulch J, Acharya M. University of California, Irvine, Irvine, CA, United States.

The CNS is an exquisitely organized tissue with intrinsic morphologic and biochemical hierarchy that defines the complexity of the cognitive networks that impact CNS functionality. Structural and synaptic plasticity are necessary elements regulating the communication of cells within the CNS, and disruptions to these processes caused by acute or chronic injury, stress, age, or disease can have adverse and long-lasting consequences to cognitive health. Radiation- and chemotherapy-induced neural stem cell depletion in the dentate gyrus of the hipppocampus is causal to the inhibition of neurogenesis found after exposure, and contributory to impaired cognition. Resultant oxidative stress and neuroinflammation that persist from weeks to months and possibly years following exposure remodel the compromised microenvironment with a range of multifaceted consequences. Recent studies using *in vitro* and *in vivo* model systems have revealed that irradiation elicits a marked and persistent oxidative stress and structural plasticity in newly born and more mature neurons throughout the brain. These changes show no sign of reversal and occur throughout different regions of the brain. This talk will focus on the implications of cell loss, dendritic alterations and reduced spine densities as they apply to functional endpoints in the CNS exposed to radiation or chemotherapy. Furthermore, we will highlight our recent work describing the use of stem cell transplants to ameliorate radiation or chemotherapy-induced cognitive dysfunction, as well as describe the mechanistic basis for the beneficial effects of engrafted cells in the brain.

S27

Formaldehyde Exposure and Amyotrophic Lateral Sclerosis. Weisskopf MG. Harvard School of Public Health, Boston, MA, United States.

Introduction: Several lines of evidence suggest a role for environmental factors in the risk of amyotrophic lateral sclerosis (ALS), but there is little data from large prospective investigations. Methods: We prospectively assessed the relation between self-reported regular exposure to 12 different chemical classes, including formaldehyde, and ALS mortality among over 1 million participants in the Cancer Prevention Study II of the American Cancer Society. Follow-up from 1989 through 2004 identified 617 ALS deaths among men and 539 among women. We calculated adjusted rate ratios (RR) using Mantel-Haenszel weights and Cox proportional hazards. Results: The only exposure with a suggestively increased RR for ALS was formaldehyde (RR: 1.34; 95% CI: 0.93-1.92). Among those reporting duration of formaldehyde exposure, there was an increasing dose-response relation with increasing years of exposure, with RRs of 1.5 (95% CI: 0.5-4.0), 2.1 (95% CI: 0.8-5.3), and 4.1 (95% CI: 2.2-7.7) for those reporting <4 years, 4-10 years, and >10 years of exposure, respectively, compared with the unexposed (p-trend=0.03). The RR for ALS among those in the eight highest formaldehyde exposure-reporting jobs was 1.3 (95% CI: 1.0-1.6) compared with those in other jobs. Discussion: We found evidence suggesting an increased risk of ALS with formaldehyde exposure. We will discuss epidemiological studies that have followed since this first report of an association with formaldehyde, including our ongoing examination of the risk of ALS with occupational exposure to formaldehyde among 1,469,478 subjects (758 ALS deaths) in the National Longitudinal Mortality Study, a US population-representative cohort.

Applied Symposium 3—Advances and Use of Transgenic Rodent Mutation Assays in Risk Assessment: Update on Recent Developments and Applications

S28

Historical Development of Transgenic Rodent Mutation Assays and Future Challenges. Lambert IB. Carleton University, Biology Department, Ottawa, ON, Canada.

Approximately 25 years ago, the first transgenic rodent (TGR) mutation model was developed by stably integrating a phage vector, containing the bacterial *lacZ* gene, into the mouse chromosome. Subsequently, a variety of TGR models have been developed, of which Muta™mouse, Big Blue®mouse and rat, the *lacZ* plasmid mouse, and the *gpt* delta mouse and rat have been used most extensively. The principal advantage of the assay is that gene mutations induced by test agents may be measured in any rodent tissue. Recently, OECD Test Guideline 488 was adopted to provide regulatory harmonization for the use of the TGR assay in assessment of somatic and germ cell mutagenicity. With the adoption of a Test Guideline, the TGR assay will find more extensive regulatory and commercial use, and experimental designs can be optimized to allow the measurement of multiple genetox endpoints in a single animal, thus minimizing animal use. Moreover, cell lines and primary cell cultures derived from the *in vivo* TGR models provide corresponding *in vitro* models that may, in some contexts, provide viable surrogates for *in vivo* studies. In addition TGR models are being increasingly used for additional types of study, including the following: 1) the assay facilitates germ cell studies that may be conducted using relatively small numbers of animals; 2) genetic modification of the existing TGR rodents may allow development of animal models for examining mutation in susceptible individuals; and 3) passive parallel sequencing provides a mechanism through which detailed mutational spectra and mechanistic data can be obtained.

S29

Advances in the Transgenic Rodent Assay for Germ Cell Mutagenicity Testing. Marchetti F, Yauk CL. Health Canada, Ottawa, ON, Canada.

There is a rekindled interest in the characterization of genotoxic effects of environmental agents on germ cells and the identification of human germ cell mutagens. The transgenic rodent (TGR) mutation assay is a method recently endorsed by the OECD for testing the ability of chemicals to induce DNA mutations *in vivo*, including those that occur in the germline. Research in our laboratory uses the Muta™Mouse model to develop improved testing methods for analyzing both germ cell mutagenicity and clastogenicity and identifying environmental factors that affect mutation frequencies in germ cells. We have used two established germ cell mutagens, N-ethyl-N-nitrosourea (ENU) and benzo[a]pyrene (BaP), to demonstrate: the presence of a saturable DNA repair mechanism in spermatogonia that is able to prevent mutations at low doses but not at high doses; that dividing spermatogonia represent a window of peak sensitivity for the induction of mutations during spermatogenesis; and, that *in utero* exposure to BaP causes mutations in sperm of F1 males. Work in support of the OECD test guideline for identifying a single time point that is effective for both somatic and germ cells suggests that analysis of cells from seminiferous tubules at the time point recommended for somatic tissue analysis provides a reasonable estimate of mutagenic effects in stem cell spermatogonia, but greatly underestimates the response in dividing spermatogonia. Finally, we have integrated next generation sequencing into the TGR assay to rapidly sequence mutants and establish mutation spectrum across multiple tissues allowing for a better comparison of genotoxic effects among tissues.

S30

Development of a Transgenic, Hairless Albino Mouse Model for Testing UV-induced Circadian Clock in the Skin. Manjanatha MG[1], Shelton SD[1], Gaddameedhi S[2], Sancar A[2], Boudreau M[1]. [1]National Center for Toxicological Research, Divisions of Genetic and Molecular Toxicology and Biochemical Toxicology, US FDA, Jefferson, AR, United States, [2]University of North Carolina School of Medicine, Department of Biochemistry and Biophysics, Chapel Hill, NC, United States.

Recently, a circadian rhythmicity associated with UV exposure was demonstrated in mouse skin. Mice exposed to UV in the morning displayed a decreased latency and a five-fold increase in the multiplicity of skin tumors, when compared to mice that were exposed to UV in the evening. To test if UV-induced mutagenicity shows a similar circadian rhythmicity, we developed a transgenic hairless albino (THA) mouse by crossing the *gpt*-delta transgenic mouse with the SKH-1 hairless mouse and exposed groups of male THA mice in the morning or evening for three consecutive days to ultraviolet-B (UVB) irradiation emitted from FS-40 HO fluorescent lamps at exposure levels of 0.00, 6.85, or 13.70 $mJ{\cdot}CIE/cm^2$. Exposure levels were measured with a UV dosimeter that was calibrated to a NIST-traceable standard. Mice were sacrificed two weeks after the last exposure, and dorsal skin DNA was extracted for *gpt* and Spi^- mutant frequency (MF) evaluations. UVB exposures at 6.85 and 13.70 $mJ{\cdot}CIE/cm^2$ induced significant increases (10-15-fold) in the *gpt* MFs over background ($22 \pm 3 \times 10^{-6}$) and induced significant increases (4-6-fold) in the Spi^- MFs over background ($8 \pm 1.5 \times 10^{-6}$); however, there was no significant difference in the *gpt* or Spi^- MFs between the groups of mice irradiated in the morning and evening. These results suggest a lack of circadian effect in skin as measured by the transgenic reporter genes. Efforts are underway to measure directly in skin UVB-signature mutations in the *p53* gene by methods such as next generation sequencing and allele-specific-PCR.

S31

Accumulation of Spontaneous Point Mutations and Deletions with Aging in *gpt* Delta Transgenic Rodents. Masumura K[1], Toyoda-Hokaiwado N[1], Osugi N[1], Honma M[1], Ishii Y[2], Umemura T[2], Nishikawa A[3], Nohmi T[3]. [1]Division of Genetics and Mutagenesis, National Institute of Health Sciences, Tokyo, Japan, [2]Division of Pathology, National Institute of Health Sciences, Tokyo, Japan, [3]Biological Safety Research Center, NIHS, Tokyo, Japan.

We are exposed to endogenous and exogenous mutagens throughout lifetime. DNA damages and DNA replication errors induce gene mutations. Although gene mutations are thought to accumulate with age, however, effects of aging on different types of mutations such as point mutations and deletions have not been well studied. The *gpt* delta transgenic rodents are unique models to efficiently detect point mutations (*gpt*) as well as deletions (Spi^-). Spontaneous mutations were analyzed for their accumulation with aging. Male C57BL/6J *gpt* delta mice were fed basal diet for 2 years. In the liver, the *gpt* mutation frequencies significantly increased with age up to 3 times until 78 weeks. Spi^- deletion frequencies were constant from week 4 to week 78 but significantly increased 2 times at week 104. In the testis, the *gpt* mutation frequencies increased 3 times only at week 104 although the increase was not statistically significant. Spi^- deletion frequencies didn't increase with age. These results suggest that the point mutations and deletions accumulate with aging in a different manner. In addition, F344 *gpt* delta rats were fed basal diets for 2 years. In the liver of the aged rats at week 104, *gpt* mutation frequencies increased 2~3-fold higher than those at week 19. Interestingly, G:C to T:A transversions were induced in the aged male rats more than those in the female. Influences of clonal mutants observed in the aged animals are also discussed.

S32

Genotoxicity Testing: *In Vitro* Methods Derived from Transgenic Mice. Luijten M[1,2], Zwart EP[1], Cox J[3], White PA[3], van Benthem J[1]. [1]National Institute for Public Health and the Environment, Centre for Health Protection, Bilthoven, Netherlands, [2]Leiden University Medical Center, Department of Toxicogenetics, Leiden, Netherlands, [3]Health Canada, Environmental Health Sciences and Research Bureau, Ottawa, ON, Canada.

At the end of last century, transgenic animals carrying an exogenous reporter gene were developed to fill a gap in the strategy for genotoxicity testing. Sufficient assays for detecting gene mutations or chromosomal aberrations in mammalian cells were available to assess the genotoxic potential of a given chemical. However, a well-validated *in vivo* gene mutation test to confirm positive results from *in vitro* gene mutation tests was lacking. Various transgenic models in rats and mice have been developed: the *lacZ* bacteriophage mouse (MutaMouse); the *lacZ* plasmid mouse; the *gpt* delta (*gpt* and Spi-) mouse and rat, and the *lacI* mouse and rat (Big Blue). These animal models have in common that they contain many copies of the transgene in a shuttle vector or plasmid, which are transmitted by the germ cells to the offspring, and thus are present manifold in all cells including germ cells. Assays using these transgenic rodents have now been adopted as OECD test guideline 488. Overall, these models have been demonstrated to be very useful for the assessment of genotoxicity and even for carcinogenicity, as the sensitivity and positive predictivity of the transgenic assays for carcinogenicity are high. To take advantage of the beneficial characteristics of the transgenic models, several *in vitro* assays derived from these transgenic rodents have been generated. The cell types used in these assays range from mouse embryonic fibroblasts to primary hepatocytes. An overview on currently available *in vitro* methods derived from transgenic models for the assessment of genotoxicity will be provided.

Basic Symposium 3—Environmental Epigenetics, Epigenotoxic Assays, and Regulation

S33

Regulation of Chemicals Based on Epigenetic Liability: Are We There Yet? Goodman J. Michigan State University, East Lansing, MI, United States.

Epigenetics refers to heritable mechanisms superimposed on DNA base sequence that regulate gene expression (thus, the term epi- (Greek: over, above) genetics). Methylation at the 5-position of DNA-cytosine (5mC, the 5th base), the histone code and non-coding RNAs contribute to epigenetic regulation of transcription. Additionally, ten-eleven translocation (TET) proteins can metabolize 5mC to 5-hydroxymethylcytosine (5hC, the 6th base), 5-formylcytosine (5fC, the 7th base) and 5-carboxycytosine (5caC, the 8th base) through three consecutive oxidation reactions. 5caC can be excised from DNA by thymine-DNA glycoslase. Thus, the oxidized cytosines are intermediates in the process of DNA demethylation. Furthermore, these oxidized 5mC derivatives might play an epigenetic role(s) that is not exactly the same as 5mC. Interest in understanding how epigenetic changes might underlie toxicity/disease (as a causative or susceptibility factor), including adverse transgenerational effects, is increasing. This presentation will provide an overview of epigenetics, emphasizing how the parameters involved are integrated and interact in a coordinated fashion to affect transcription. Potential effects of nutrition, inflammation and DNA adducts will be explored. Additionally, the relationship(s) between metabolism and epigenetics will be considered. This will provide a basis for discussing issues to contemplate when considering epigenetics with regard to mode of action and safety evaluation. Might some alterations be beneficial? Within the overall context of this presentation, the importance of fundamental principles of toxicology, e.g., the need to focus on dose-response, criteria for a maximum dose, normal variability, change vs. an adverse effect and rodent to human extrapolation will be emphasized.

S34

Potential Mechanism for the Transfer of Environmental Exposures to Epigenetic Change. Turker MS[1], Lu Y[2], Glazer PM[2], Raber J[1], Impey S[1]. [1]Oregon Health & Science University, Portland, OR, United States, [2]Yale University, New Haven, CT, United States.

Environmental exposures can alter gene expression by inducing mutations via mechanisms that are well described. Far less is known, however, about how environmental exposures lead to epigenetic changes in gene expression. We have been testing the hypothesis that prolonged perturbations in gene expression resulting from environmental exposures trigger epigenetic change. We first tested this hypothesis with cultured cells containing the tet-off promoter and showed that prolonged promoter repression caused stable silencing associated with changes in histone acetylation, histone methylation, and DNA methylation. We next examined whether this model would work with two tumor suppressor promoters commonly silenced in cancer, *BRCA1* and *MLH1*. Both are repressible under hypoxic conditions. We again found that promoter repression triggered promoter silencing, and also showed that inhibition of histone deacetylation or histone demethylation prevented silencing of the *BRCA1* or *MLH1* promoters, respectively. We then asked whether our model works *in vivo* by treating mice with ionizing radiation to induce a prolonged repair response. DNA methylation changes were examined in the hippocampus and left ventricle as long as 5 months after exposure. We observed tissue-specific differences in DNA methylation that mapped to regions associated with neurogenesis in the hippocampus and vascular development in the left ventricle, in addition to responses that were common to both tissue types. These DNA methylation changes persisted *in vivo* for at least 5 months after a single exposure. In sum, our results support the hypothesis that environmental exposures can trigger epigenetic change as a result of perturbed gene expression.

S35

Epigenetic Assays: Updates, Benefits, and Limitations. Klein CB. New York University School of Medicine, Tuxedo, NY, United States.

The burgeoning field of environmental epigenetics relies on a multitude of assays (*in vitro*, *in silico*) and *in vivo*, to assess a variety of epigenetic endpoints following exposures to toxicants and environmental agents. Epigenetic assessments of DNA methylation and histone modification status can be made on a genome-wide basis, or on a gene-specific basis, often with differing outcomes depending in part on the dose, the duration of exposure, the cell type and the snapshot of time at which the assessments were made. An update on epigenetic assays, benefits and limitations will be presented, with an emphasis on assays that are cell based and informative using human-exposure relevant doses of several recognized epigenome modifiers, including carcinogenic metals, arsenic, and other examples.

S36

Diverse Epigenetic Enzymes Empower Regulated Gene Expression. Archer TK. National Institute of Environmental Health Sciences, Molecular Carcinogenesis Laboratory, Research Triangle Park, NC, United States.

The precise architecture of various DNA regulatory elements within the hierarchy of chromatin structures controls the access and activity of transacting factors, both protein and RNA. This assembly of DNA with histones into chromatin provides a mechanism to regulate gene expression in time and space. Epigenetic marks within DNA and histones allow regulatory information to act during development and across generations. The enzymes that mediate these processes are diverse and multifunctional, capable of posttranslationally modifying DNA and histones as well as remodeling the chromatin architecture. Among these enzymes the human SWI/SNF or BRG1 remodeling complex is particularly important with clear roles in gene transcription, signal transduction, development and cancer. In recent studies we focus on the contribution that sub-domains in the BRG1 N terminus makes if protein partners outside the normal BRG1 associated Factors (BAFs) to initiate steroid receptor mediated transcription. These include interactions with proteins normally associated with DNA repair and responses to external environmental signals. These findings highlight the intimate connections between chromatin remodeling and epigenetics required for regulated gene transcription.

S37

Relationships between Mercury Exposure, DNA Methylation, and Cardiometabolic Risk Factors. Goodrich JM. University of Michigan School of Public Health, Ann Arbor, MI, United States.

Characterization of the interplay between toxicants, epigenetic modifications, and disease outcomes is necessary to improve risk assessment of common exposures. Mercury (Hg) is a prevalent toxicant that humans are exposed to via fish consumption (as methylmercury) and dental amalgam (as inorganic Hg). Emerging evidence suggests that Hg alters the epigenome, though the implications of these changes for toxicity and disease is unknown. Mercury impacts various health outcomes including early indicators of cardiometabolic disorders. This study hypothesizes that methylmercury and inorganic Hg both alter DNA methylation patterns and associate with adverse cardiometabolic outcomes. Further, DNA methylation changes are expected to mediate relationships between Hg and cardiometabolic risk factors. American dental professionals with occupational (inorganic Hg) and environmental (methylmercury) exposures were recruited to address the hypotheses. Hair, blood, and urine mercury levels exhibited a range that overlaps with Hg levels of the US population. The influence of Hg on DNA methylation was assessed with candidate gene and discovery approaches. DNA methylation was quantified via bisulfite sequencing using blood leukocyte DNA (n=410) and saliva DNA (n=209) at repetitive elements and selenoprotein p1. In 48 males, blood leukocyte DNA methylation was quantified at >450,000 CpG sites via the Infinium BeadChip. Statistical analyses revealed loci that are differentially methylated by Hg levels and also cardiometabolic outcomes associated with Hg including elevated glycated hemoglobin and cholesterol. By integrating DNA methylation, health outcome, and Hg biomarker data, results are expected to improve understanding of the true risk for development of cardiometabolic disorders from Hg exposure.

Applied Symposium 4—Toxicogenomics in the Assessment of Mutagenicity and Carcinogenicity: Implications for Risk Assessment (Part 1)

S38

Introduction to the Session and a Review of the Literature. Waters MD. Integrated Laboratory Systems, Inc., Research Triangle Park, NC, United States.

Toxicogenomics (TGx) has three principal goals: 1) to understand the relationship between environmental exposures and human disease, 2) to identify predictive biomarkers or signatures of exposure to toxic substances, and 3) to elucidate molecular mechanisms of toxicity. The Society has had similar goals for conventional bioassays used in the assessment of mutagenicity and potential carcinogenicity. This session will document the current status of TGx methods as they have been applied in the fields of predictive carcinogenicity testing, mode of action and safety evaluation, and in cancer risk assessment. This introductory presentation will trace the beginnings of the field of TGx and its methodology, from the turn of the century to the present, introducing its application in the evaluation of mode of action, safety evaluation, and risk assessment. Research over the past decade has demonstrated that TGx methods of various types can used together with conventional methods to clearly discriminate genotoxic, nongenotoxic, and other modes of action, as a function of dose. Thus, TGx can quickly inform safety evaluation regarding potential mechanisms of conventional outcomes, such as positive responses in *in vitro* cytogenetics assays, and can provide essential dose-response information to enable the ascertainment of the sequence of key events in a putative mode of action, as may be required in cancer risk assessment. The session also will address current and potential future TGx research using stem cells as well as new bioinformatics methods as they are being applied in the evaluation of environmental toxicants and in drug discovery.

S39

Toxicogenomics *In Vitro*: Gene Expression Signatures for Discriminating Genotoxic from Nongenotoxic Mechanisms. Buick J[1], Moffat I[1], Swartz CI[2], Williams A[1], Hyduke D[3], Recio L[2], Fornace Jr A[4], Li H[4], Aubrecht J[5]. [1]Health Canada, Ottawa, ON, Canada, [2]Integrated Laboratory Systems, Inc., Research Triangle Park, NC, United States, [3]Utah State University, Logan, UT, United States, [4]Georgetown University, Washington DC, United States, [5]Pfizer, Mystic, CT, United States.

Although existing *in vitro* genotoxicity assays are sensitive, they lack specificity leading to high rates of false positives. The Health and Environmental Sciences Institute's (HESI) Genomics Committee is evaluating an *in vitro* genomic biomarker that classifies chemicals as genotoxic or non-genotoxic with high accuracy, and classifies known false positives as non-genotoxic. However, a major issue is the lack of metabolic competency of the human TK6 cell line used. We demonstrate that the biomarker is also effective in TK6 cells co-treated with chemical agents in the presence of different metabolic activation systems (S9). We exposed TK6 cells to increasing concentrations of genotoxic (benzo[a]pyrene, BaP; aflatoxin B_1, AFB_1) and nongenotoxic (dexamethasone, DEX and phenobarbital, PB) agents in the presence of various types of rat S9 for 4h. Cells were collected 0, 4 and 20h post-exposure. Transcriptome profiles (generated with Agilent microarrays) for each chemical were classified using the biomarker. The data were aligned against HESI's chemical training set to test the predictivity of the biomarker in the presence of S9. Genotoxic concentrations of BaP and AFB_1 were correctly classified at all three time points, whereas DEX was correctly classified as non-genotoxic at all time points. The high concentration of PB was incorrectly classified as genotoxic at 24h, indicating confounding effects of cytotoxicity at late time points that may lead to misclassification. Potential misclassification also occurs in the presence of very high concentrations of S9. We propose that this approach has the potential to add significant value to the existing genotoxicity testing battery.

S40

A Large-Scale Toxicogenomic Benchmark Dose Analysis and Resource. Auerbach S. Division of the National Toxicology Program, NIEHS, Research Triangle Park, NC, United States.

The field of toxicology is transitioning to the use of metrics obtained from methods such as toxicogenomics, in order to characterize hazards associated with chemical exposure. One of the most significant challenges is to familiarize stake holders with the metrics gleaned from toxicogenomic-based benchmark dose analysis. In order to facilitate the transition, we have capitalized on the EPA's BMDS software to model all toxicogenomics data from the Open TG-Gates database, aggregated at a biological pathway level. The results of this analysis were then used to create a user interface where stake holders can interact with the results of analysis. An overview of the analysis approach, interface, and biological insights taken from the analysis will be discussed.

S41

Toxicogenomics and Dose-Response: Applications to Risk Assessment. Thomas RS. US Environmental Protection Agency, Research Triangle Park, NC, United States.

Current challenges facing chemical risk assessment are the time and resources required to meet the data standards necessary for a published assessment and the incorporation of modern biological information. The integration of toxicogenomics into the risk assessment paradigm may address both challenges by providing an efficient means to quantitatively and comprehensively evaluate molecular changes resulting from chemical exposure. To assess the value of toxicogenomics in chemical risk assessment, a series of studies was performed. In the first study, mice were exposed for 13 weeks to multiple concentrations of five chemicals that were positive in a cancer bioassay. In a second study, rats were exposed with time to multiple concentrations of six chemicals with published risk assessments. In both studies, histological changes were evaluated and transcriptional microarray analysis was performed on the target tissues. Histological and the tumor responses were analyzed using benchmark dose (BMD) methods to identify noncancer and cancer points-of-departure. Dose-related changes in gene expression were also analyzed using a BMD approach. The transcriptional BMD values showed a high degree of correlation with apical responses for specific pathways and many of the correlated pathways have been implicated in relevant disease pathogenesis. Importantly, transcriptional BMDs for even the most sensitive pathway were on average less than three-fold different than traditional apical BMDs for both cancer and non-cancer endpoints suggesting that transcriptomic changes in signaling pathways can be used to estimate noncancer and cancer points-of-departure for use in quantitative risk assessments. This abstract does not necessarily reflect US EPA policy.

S42

Developing Gene Signatures for Developmental Toxicants in Human Embryonic and Induced Pluripotent Stem Cells. Recio L[1,2], Phillips K[2], Phillips S[2], Swartz C[1], Hobbs C[1]. [1]ILS, Research Triangle Park, NC, United States, [2]ILS Genomics, Morrisville, NC, United States.

In utero exposure to environmental chemicals or drugs can have an impact on the developing fetus but also later in adult life by disrupting the epigenetic developmental programs that are associated with for example puberty and aging. The currently accepted regulatory test for identifying potential teratogens involves the use of thousands of animals, is costly, takes two years to complete, and shows poor concordance (~60%) for human teratogens. Human embryonic stem (hES) cells reflect a unique biological system that cannot be represented by any other cell type used in toxicology. We are focusing on developing and validating a rapid toxicogenomics-based signature profiling approach to *in vitro* developmental toxicology screening in a human-relevant assay using the cell type at the origin of human development, the hES cell. For these studies we are using hES H9 cells and iPS cells, 2-4 days of exposure and samples for gene expression are collected 24 hrs post exposure. Hierarchical clustering of Affymetrix array data from preliminary studies with 4 teratogens (retinoic acid, busulfan, methotrexate, valproic acid) and 3 nonteratogens (retinol, folic acid, penicillin) showed that two classes of agents can be distinguished. We are extending these studies to a larger number of agents to define a potential gene signature(s) for developmental toxicant in hES cells. hES cells are a unique biological system for the development of a biologically relevant signature profile based on dysfunction of the highly regulated genome and epigenome circuitry that maintains stem cell functions.

Basic Symposium 4—Watching DNA Repair: From Single Molecules to Living Cells

S43

Watching Base Excision Repair Glycosylases Scan for Damage. Wallace SS. University of Vermont, Burlington, VT, United States.

The first step in Base Excision Repair is catalyzed by DNA glycosylases that both locate and remove base lesions. Since these lesions often differ only slightly from their normal counterparts and are present in a sea of undamaged bases, this recognition process is the most difficult step in the pathway. To examine the search process, we used a single-molecule TIRF assay to image quantum dot-labeled glycosylases interacting with lambda DNA molecules suspended between silica beads. Bacterial Nth, Fpg and Nei, members of two structural families, exhibit a similar diffusive search mechanism: rotational diffusion along the DNA molecule ranging from very slow to faster diffusion. Structural studies showed three amino acids to be inserted into the DNA helix after the damaged base was flipped out into the glycoslyase's binding pocket. When we mutated one of these to an alanine, the slow diffusive behavior observed with the wild type glycosylases was no longer present suggesting that this wedge amino acid was involved in the damage search. When we examined the behavior of these same glycosylases on damage-containing DNA, we observed the glycosylases to stop upon encountering a damage. The similarities and differences between the search behaviors of the bacterial glycosylases and the behaviors of human OGG1, APE1 and MUTYH will also be discussed. Our data show that glycosylases use facilitated diffusion to rotate around the DNA molecule and employ a wedge residue to interrogate the DNA for damage. When the glycosylase locates a damage, it stops to remove it. NIH P01 CA098993.

S44

Single-Molecule Imaging Reveals DNA-Binding Properties of Cohesin Proteins SA1 and SA2. Lin J[1], Kaur P[1], Chen H[2], Countryman P[1], Roushan M[1], Flaherty D[3], Brennan E[1], You C[4], Piehler J[4], Riehn R[1], Tao YJ[2], Wang H[1]. [1]Physics Department, North Carolina State University, Raleigh, NC, United States, [2]Department of Biochemistry and Cell Biology, Rice University, Houston, TX, United States, [3]Genetics Department, North Carolina State University, Raleigh, NC, United States, [4]Division of Biophysics, Universität Osnabrück, Osnabrück, Germany.

The cohesin complex plays a crucial role in accurate chromosome segregation, organization of interphase chromatin, DNA replication, and post replicative DNA repair in part by promoting DNA-DNA pairing. The core cohesin subunits consist of a tripartite ring and the fourth core subunit Scc3/SA. In somatic vertebrate cells, SA can be either SA1 or SA2, which have distinct functions. SA1 is significantly enriched at promoters sites, while SA2 prefers intergenic regions. SA1 and SA2 promote cohesion at telomeres and centromere, respectively. While these results demonstrated unique roles that SA1 and SA2 play, the underlying mechanisms are poorly understood. To better understand the functions of SA1 and SA2, we used single-molecule imaging platforms including atomic force microscopy (AFM) and electrostatic force microscopy (EFM) imaging, fluorescence imaging of quantum dot-labeled protein on DNA tightropes, and nanochannel confined DNA. AFM and fluorescence imaging reveal that SA1 carries out 1-D sliding on DNA, binds specifically to telomeric sequences and pauses on DNA with telomeric and promoter sequences. SA2 has preference for DNA ends and can bridge a DNA end to a second piece of duplex DNA. Both SA1 and SA2 mediate DNA-DNA pairing and facilitate DNA loop formation and EFM reveals DNA paths inside SA-DNA complexes. Importantly, DNA loops mediated by SA1 and SA2 inside nanochannels are mobile. We propose that 1-D sliding and sequence dependent pausing by SA1 provide binding specificity and stability during the cohesion process at telomeres. SA1 and SA2 mediated DNA-DNA pairing facilitates cohesion process in addition to the ring structure.

S45

Damage and Repair at Telomeres. Opresko PL, Parikh D, Pope-Varsalona H, Lormand J, Murphy C. Department of Environmental and Occupational Health, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA, United States.

Telomeres preserve genome stability, survival, and proliferation on a cellular level and prevent degenerative diseases and cancer on an organism level. Dysfunctional telomeres trigger cell senescence or cause chromosomal instability. We are investigating the impact of telomeric DNA damage on telomere structure and function, and the cellular pathways that preserve telomeres after genotoxic exposures. We propose that telomeres are highly sensitive to DNA lesions that interfere with replication because homologous recombination and DNA double strand break repair, which can restore stalled or broken replication forks, are normally suppressed at telomeres. Using a novel assay we demonstrate that ultraviolet light exposure of human cells causes the formation of both cyclobutane pyrimidine dimers (CPD) and 6-4 pyrimidone photoproducts (6-4 PP) at telomeres. CPDs in telomeres are removed slowly similar to the bulk genome. By analyzing individual telomeres we discovered that UV irradiation of human cells induces telomere aberrations associated with failures in telomere replication. UV-induced telomere defects were dramatically increased in cells lacking translesion DNA polymerase η, which normally bypasses CPDs during replication. Oxidative stress has also been reported to induce telomere loss and aberrations, although the mechanism is poorly understood. We developed a novel approach for selectively generating reactive oxygen species at the telomeres so that we can delineate the impact of telomeric oxidative DNA damage on telomere replication and maintenance. Understanding how telomeres are damaged and repaired will be useful for advancing interventions aimed at preserving telomeres to maintain healthy cells and tissue after genotoxic exposures.

S46

Cdt2-Mediated XPG Degradation Promotes Gap-Filling DNA Synthesis in Nucleotide Excision Repair. Wang Q-E, Han C, Zhao R, Qian J, Sharma N, Wani G, He J, Zhu Q, Wani A. The Ohio State University, Columbus, OH, United States.

Xeroderma pigmentosum group G (XPG) protein is a structure-specific repair endonuclease, which cleaves DNA strands on the 3' side of the DNA damage during nucleotide excision repair (NER). In addition, XPG plays a crucial role in initiating DNA repair synthesis through recruitment of PCNA to the repair sites. However, the fate of XPG protein subsequent to the excision of DNA damage has remained unresolved. Here, we show that XPG is degraded through proteasome-mediated proteolysis upon induction of bulky lesions from exposures to UV irradiation and cisplatin. NER process is required for XPG degradation because both UV and cisplatin treatment-induced XPG degradation is compromised in NER-deficient XP-A, XP-B, XP-C, and XP-F cells. In addition, the NER-related XPG degradation requires Cdt2, a component of an E3 ubiquitin ligase, $CRL4^{Cdt2}$. Micropore local UV irradiation and *in situ* Proximity Ligation assays demonstrated that Cdt2 is recruited to the UV-damage sites and interacts with XPG in the presence of PCNA. Importantly, Cdt2-mediated XPG degradation is crucial to the subsequent recruitment of DNA polymerase δ and DNA repair synthesis. Collectively, our data supports the idea of PCNA recruitment to damage sites in conjunction with XPG, recognition of the PCNA-bound XPG by $CRL4^{Cdt2}$ for specific ubiquitylation and protein degradation. Thus, XPG removal clears the space needed at the damage site for the subsequent recruitment of DNA pol δ and initiation of DNA synthesis. This work was supported by grants from NIH.

S47

Watching Nucleotide Excision Repair Proteins: One Molecule at a Time. Van Houten B[1], Kong M[1], Liu L[1], Ghodke H[1], Kad N[2], Min J-H[3], Watkins S[4]. [1]Department of Pharmacology and Chemical Biology, University of Pittsburgh School of Medicine and University of Pittsburgh Cancer Institute, Pittsburgh, PA, United States, [2]Department of Biophysics, University of Kent, Canterbury, United Kingdom, [3]Department of Chemistry, University of Illinois at Chicago, Chicago, IL, United States, [4]Center for Biologic Imaging, University of Pittsburgh School of Medicine, Pittsburgh, PA, United States.

Nucleotide excision repair is a highly conserved DNA repair pathway that is important for the removal of a wide variety of helix-distorting DNA lesions, including UV-induced photoproducts. In order to interrogate the individual steps of bacterial and eukaryotic NER, we have employed two complementary single-molecule methods: atomic force microscopy and fluorescence microscopy. In the latter technique using DNA tightropes, NER proteins responsible for initiating NER are labeled with fluorescent quantum dots and their interactions with DNA are observed in real time at 10 frames per second, under oblique angle illumination. During human repair, UV-induced photoproducts are first recognized in chromatin by UV-DDB and then handed off to XPC-HR23B, which helps guide the global genome DNA repair factors to this site. We have been investigating the interaction of *Saccharomyces cerevisiae* homolog, Qdot-labeled Rad4-Rad23 with long "DNA damage arrays" (> 40 kb) containing one lesion every 2 kb. We have labelled damage sites with Qdots and found that Rad4-Rad23 binds tightly to fluorescein-dT adducts, but shows constrained motion at sites of UV-induced cyclobutane pyrimidine dimers. Removal of β-hairpin 3, the motif that inserts into DNA to facilitate damage recognition, causes increased mobility and random diffusion of Rad4-Rad23 on DNA. These data suggest that Rad4-Rad23 probes DNA through dynamic insertion of the β-hairpin 3, and the strength of this interaction is dependent upon the nature of the DNA lesion; weak interactions allow the complex to diffuse away from the damaged site, while partial β-hairpin insertion results in constrained motion around the damage site.

S48

Live Cell Imaging of Chromatin Remodeling at Lesion-Stalled Transcription. Vermeulen W. Erasmus MC, Rotterdam, Netherlands.

DNA damage disturbs replication and transcription and may cause cancer and ageing. The DNA damage response (DDR), including several DNA repair pathways, counteracts the consequence of DNA damage and is controlled by post-translational modifications, such as phosphorylation and ubiquitylation. Within a dedicated DNA damage-induced proteomic-ubiquitylation screen coupled to live cell imaging we identified and analyzed novel transcription-coupled repair (TCR) factors. Within subsequent proteomic network analyses we identified TCR-associated chromatin remodeling factors and chromatin-bound mRNA maturation factors and further focused on the damage-induced dynamic changes of chromatin-bound proteins. We have developed imaging procedures to allow the analysis of TCR and transcript maturation factors kinetics and to measure the dynamics of chromatin and chromatin-remodelers at lesion-stalled transcription in living mammalian cells. With this combined proteomic and live cell imaging system we disclosed a dynamic choreography between DNA repair, transcription, and chromatin modifiers.

Applied Symposium 5—Toxicogenomics in the Assessment of Mutagenicity and Carcinogenicity: Implications for Risk Assessment (Part 2)

S49

Toxicogenomic Analysis of Formalin-Fixed Paraffin-Embedded (FFPE) Samples: A Case Study of the Liver Carcinogen Furan. Webster AF[1,2], Williams A[1], Recio L[3], Yauk CL.[1]. [1]Health Canada, Ottawa, ON, Canada, [2]Carleton University, Ottawa, ON, Canada, [3]ILS, Research Triangle Park, NC, United States.

The use of toxicogenomics in chemical risk assessment is gaining popularity because it produces data quickly, using fewer animals, and at a lower cost than the 2-year cancer bioassay. Our case study of the hepatocarcinogen furan anchored molecular data at three weeks to known histophathological outcomes at two years. Toward the goal of reducing experimental animal usage, we propose that additional retrospective studies could be conducted using existing, archival formalin-fixed paraffin-embedded (FFPE) tissue blocks. To this end we performed microarray analysis of livers from mice that had been sub-chronically exposed to a carcinogenic dose of furan. Each liver was divided and preserved by freezing at -80°C, or fixing in formalin for 18 hours or 3 weeks followed by storage in paraffin at room temperature. The gene expression (GEx) profiles of paired high quality fresh-frozen and degraded FFPE samples were compared. GEx profiles of each dataset were highly correlated with mouse studies that were consistent with furan's mode of action, including liver cancer and liver regeneration. Key molecular events to the furan mode of action were enriched across samples, such as the Nrf2 oxidative stress response pathway. Ultimately data from toxicogenomic analysis of FFPE samples archived from previous cancer bioassays could be used to build: 1) a weight of evidence for the use of toxicogenomics in chemical risk assessment, and 2) produce a training set against which GEx profiles of new substances could be compared, while reducing, if not eliminating, the additional animal testing and the associated costs.

S50

Integration of Genomic Biomarkers for Xenobiotics with Positive Findings in Chromosome Damage Assays. Li H-H[1], Hyduke DR[3], Yauk CL[4], Aubrecht J[2], Fornace Jr. AJ[1]. [1]Georgetown University, Washington, DC, United States, [2]Pfizer Inc., Groton, CT, United States, [3]Utah State University, Logan, UT, United States, [4]Health Canada, Ottawa, ON, Canada.

Despite scientific progress in understanding cancer mode of action, experimental approaches for assessing oncogenic risk associated with exposure to chemicals rely mainly on traditional methods. The currently used *in vitro* genotoxicity testing battery does not offer sufficient mechanistic information for assessing oncogenic risk to humans. Recent advances in molecular biology and bioinformatics have enabled interrogation of cellular responses to chemical exposure at the genomic level. This approach has revealed molecular pathways and networks that are mechanistically involved in chemical carcinogenesis. We have constructed a reference database containing gene expression profiles of 28 model agents with a broad range of toxic mechanisms. A genotoxic-signature (biomarker) comprising 65 genes, TGx28.65, has been identified from this reference database by using the nearest shrunken centroids (NSC) algorithm. TGx-28.65 discriminates genotoxic from non-genotoxic agents with 100% accuracy after 10-fold cross validation. In a subsequent biomarker validation study, 42 chemicals were selected to test TGx-28.65's performance on genotoxicity identification. Interestingly all except one out of eleven chemicals with false positive results in *in vitro* chromosome aberrant assay were classified as "nongenotoxic" using TGx-28.65. Advanced bioinformatics analysis is in progress to further identify gene markers that significantly correlate with other toxic mechanisms. Applications of the genomic biomarker in drug development and its potential limitations will be discussed.

S51

Bioinformatics Applications Reveal Gene Regulation Complexities in Response to Toxicant Exposures. Bushel PR[1], Wang C[3], Gong B[2], Thierry-Mieg J[4], Thierry-Mieg D[4], Xu J[2], Fang H[2], Kreil DP[5,6], Megherbi D[7], Li J[8], Paules RS[1], Shi L[2], Auerbach SS[1], Tong W[2]. [1]National Institute of Environmental Health Sciences, Research Triangle Park, NC, United States, [2]National Center for Toxicological Research, Jefferson, AR, United States, [3]Loma Linda University, Loma Linda, CA, United States, [4]National Center for Biotechnology Information, Bethesda, MD, United States, [5]Boku University, Vienna, Austria, [6]University of Warwick, Coventry, United Kingdom, [7]University of Massachusetts, Lowell, MA, United States, [8]Kelly Government Solutions, Durham, NC, United States.

The central dogma of molecular biology presents the flow of genetic information from DNA to RNA to proteins. The regulation of genes is one of several mechanisms controlling the production of RNA and proteins. Advances in molecular biology, genomics and bioinformatics paint a more refined picture of the level of complexity involved in gene regulation. Using a toxicogenomics gene expression dataset acquired from the livers of rats exposed to agents specific for their mode of action, toxicant-responsive events such as alternative splicing, signaling pathway regulation and 3' untranslated region shortening were ascertained through the application of cutting-edge bioinformatics approaches. For example, utilization of a Poisson hidden Markov model detected 345 genes with usage of an alternative polyadenylation site in samples treated with phenobarbital (PHE). These genes over-represent ubiquitin-mediated proteolysis and components of spliceosomes. In addition, a probabilistic framework that quantitates the expression level of alternatively spliced genes identified 408 isoforms that were differentially spliced between control and PHE-treated samples and were mainly associated with SH3 domain binding. Furthermore, based on gene sets that share co-regulation of gene expression, several signaling pathway mediators were revealed from the perturbations and suggest dysregulation of the ectodysplasin A receptor (EDAR) transduction pathway. By applying innovative bioinformatics to the analysis of toxicogenomics data, the intricacies underlying gene regulation in response to toxicant exposures were elucidated and reveal the underlying mechanistic/molecular consequences from the exposure to adverse agents.

S52

Reading the Toxicogenomic MAP (TXG-MAP): Modular Gene Expression Changes Associated with Pathogenesis. Stevens JL. Lilly Research Laboratory, Indianapolis, IN, United States.

Transcript profiling methods, such as signatures and pathway enrichment, use supervised approaches introducing bias into analyses. Weighted gene coexpression network analysis (WGCNA) allows unsupervised assembly of gene expression "modules" based on significant coregulated behavior. We have used WGCNA and the Iconix rat liver database to construct the TXG-MAP, a relationship model for gene expression modules associated with pathogenesis (MAP). Module "eigengene" scores reflect the module response to chemical treatment. Pair-wise comparisons positions each module on the TXG-MAP in a simplified visual pharmacological similarity context. Module preservation across liver, kidney, heart, skeletal muscle, and rat primary hepatocytes suggests that up to 70% of the modules are preserved across systems. Modules were also preserved in the TG-GATES toxicogenomic database suggesting that the TXG-MAP enables translation of risk assessment across different *in vivo* and *in vitro* systems. Enrichment analysis for Gene Ontology (GO) terms and pathway information show that modules embody common biological themes that are either up or down regulated in rat liver after toxicant challenge provide mechanistic insights regarding pathogenesis *in vivo.* For example, the TXG-MAP suggests functional associations among mitochondrial metabolism, oxidative stress and protein damage, common themes in adverse outcome pathway analysis. Changes in additional biological responses modules representing sexually dimorphic function, stress response and DNA damage pathways, nutrient and bile acid metabolism, and other biological response networks are easily visualized. The TXG-MAP represents a robust method for mechanism-based risk assessment and compound classification in a "readable" format that may be translatable across biological models.

Basic Symposium 5—Topoisomerases

S53

Naturally Occurring Topoisomerase Inhibitors: The Good, the Bad, and the Bioflavonoids. Osheroff N, Ashley RE, Vann KR, Lindsey RH. Vanderbilt University School of Medicine, Nashville, TN, United States.

Human type II topoisomerases are enzymes that help regulate DNA under- and overwinding and remove knots and tangles from chromosomes. However, because they generate double-stranded breaks as part of their catalytic mechanism, these enzymes also have the capacity to fragment the genome. Because of this property, type II topoisomerases are targets for important anticancer drugs (referred to as topoisomerase II poisons), which act by increasing levels of enzyme-mediated DNA breaks. Topoisomerase II poisons also are consumed daily as part of the human diet and are prevalent in medicinal herbs. For example, bioflavonoids (such as genistein) are rich in fruits and vegetables and catechins (such as epigallocatechin gallate) are found in green tea. Recent studies also identify curcumin (from turmeric), thymoquinone (from black seed), and hydroxytyrosol, oleuropein, and verbascoside (from olive leaves and fruit) as topoisomerase II poisons. Although anticancer drugs induce topoisomerase II-mediated DNA cleavage by acting non-covalently at the enzyme-DNA interface, many dietary topoisomerase II poisons incorporate reactive groups such as quinones (thymoquinone) and act by covalently adducting the protein. Polyphenols often can be activated to quinones in the presence of oxidants. In some cases (hydroxytyrosol and oleuropein), oxidants can increase their potency >10-fold. Recent studies suggest that covalent poisons interrupt disulfide bridges that help regulate the opening/closing of the N-terminal protein gate and enhance DNA cleavage by closing the gate. If the N-terminal gate is deleted from topoisomerase II, these compounds do not enhance DNA cleavage. Supported by NIH grants GM033944 and GM065086 and NSF grant DGE-0909667.

S54

Genome Instability Induced by Eukaryotic Topoisomerase II. Nitiss JL, Rogojina A, Bartholomew S, Pant B, Gilbertson M. University of Illinois, Rockford, IL, United States.

DNA topoisomerase II (Top2) is an important target in cancer chemotherapy, and clinically active agents targeting this enzyme act by converting the enzyme into a unique DNA damaging agent. The generation of enzyme mediated damage is cytotoxic to cancer cells, but can also to genomic alterations that can lead to secondary malignancies. The mechanisms leading to topoisomerase II induced mutations remain poorly understood. We have developed a variety of tools to assess the genetic consequences of Top2 mediated DNA damage. We generated mutants of Top2 that mimic exposure of cells to agents that trap Top2 poisons termed Top2 (dir, dead in rad52). In addition to conferring lethality to repair deficient strains, top2dir mutants induce high levels of both homologous recombination and loss of function mutations. We have also used strains defective in multiple drug efflux proteins to assess the requirements for Top2 induced genome instability. We found that etoposide was a potent mutagen in wild type cells, and that overexpression of Top2 enhanced the mutagenesis, indicating that Top2 mediated the genome instability. Cells lacking Tdp1, a gene required for processing Top2 covalent complexes enhanced etoposide-induced mutagenesis, indicating that a failure to process the Top2 covalent complexes has mutagenic consequences. We have begun to explore the mutational spectrum induced by etoposide, and identified deletions, rearrangements, and an increased frequency of point mutations. These results indicate that genomic changes induced by targeting Top2 likely include both direct changes due to Top2 covalent complexes and indirect effects that likely arise from replication stress.

S55

TopoisomeraseIIβ Is Involved in Initiation of Interstrand Cross-Link Repair in Mammals. Smeaton MB[1,2], Bell EH[1], Austin CA[3], Deterding LJ[4], Tomer KB[4], Hanawalt PC[2], Miller PS[1]. [1]Department of Biochemistry and Molecular Biology, Johns Hopkins School of Public Health, Baltimore, MD, United States, [2]Department of Biology, Stanford University, Stanford, CA, United States, [3]Institute for Cell and Molecular Biosciences, Newcastle University, Newcastle upon Tyne, United Kingdom, [4]Laboratory of Structural Biology, NIEHS, Research Triangle Park, NC, United States.

Interstrand cross-links (ICLs) are a type of DNA damage that covalently tether the opposing strands of the DNA helix. ICLs are formed by some of the most commonly used chemotherapeutics, repair of which can result in resistance. ICLs can also form through endogenous mechanisms and, if left unrepaired, contribute towards aging processes. The pathways that repair ICLs are diverse, however, the proteins that initiate the repair process remain unknown. Here, we examine pathways of ICL repair available to terminally differentiated cells and the proteins involved in initiation of repair. Model, site-specific ICLs were synthesized that either fit perfectly into B-form DNA or cause a high level of helical distortion. Using mammalian cell extracts, a biochemical activity was uncovered that “unhooks” the ICL through nucleolytic incisions. The unhooking reaction was damage-specific, ATP/Mg^{2+} dependent and responded to the levels of helical distortion. In yeast and bacteria, the nucleotide excision repair (NER) pathway is responsible for the initial unhooking step in ICL repair. However, we found the unhooking activity to be independent of the NER pathway in mammalian cell extracts. Purification of the proteins responsible for unhooking was carried out through fractionation of cell extracts. Mass spec analysis of the final, active fraction identified topoisomerase IIβ. The topoII catalytic inhibitor, merbarone, was able to abolish the unhooking activity in extracts. Humans have two isoforms of topoII, α and β. Recombinant topoIIβ, but not α, was able to nucleolytically process a purified ICL substrate. Finally, using a plasmid with a site-specific ICL in a host-cell reactivation assay and reverse comet assays using melphalan, we confirm the involvement of topoIIβ in repair of ICLs *in vivo*. These findings may have significant clinical implications in chemotherapeutic strategy. M.B.S was supported by the American Heart Association through pre- and postdoctoral fellowships 0815220E and 11POST7600194.

S56

The Role of Topoisomerase Inhibition in Clastogenicity of Nonalerting Molecules. Snyder RD. RDS Consulting Services, Maineville, OH, United States.

The clastogenicity of classical topoisomerase inhibitors such as fused ring planar intercalators and fluoroquinolones has been known and at least partially understood for some time. These molecules and others, which act through binding topoisomerase directly, such as etoposide, have found therapeutic use in cancer and as antibiotics. A second widely diverse group of compounds act as catalytic inhibitors of topo. These molecules, through an equally diverse number of mechanisms block topo before it has a chance to initiate the nicking-closing function. In many cases, this can block the clastogenicity ofclassical topo inhibitors and these types of inhibitors are, thus, useful in determining if topo inhibition is the cause of observed clastogenicity. Recently, it has come to be appreciated that perhaps many clastogenic drugs not carrying structural alerts might be topo inhibitors. Some of these most likely are atypical DNA intercalating agents while others may interact with the catalytic function of topo. Computational docking studies have been applied to identifying DNA intercalators as well as molecules that interfere with ATP hydrolysis through binding to the ATP hydrolysis site of Topo II. Such studies have identified steroids, N-aryl ketones and benzimidazoles as potential topo inhibition-dependent clastogens.

Platform Abstracts

Applied Platform Session 1

1

Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) or Pyruvate Dehydrogenase (PDH) As a Target Molecule for the Toxicity Mechanism of Mono, Di, and Trihaloacetic Acids Water Disinfection By-Products (DBPs). Dad A, Jeong CH, Wagner ED, Plewa MJ. University of Illinois at Urbana, Champaign, IL, United States.

The haloaceticacids (HAAs) are the second most occurring class of water disinfection by products (DBPs) in chlorinated water. They are mutagenic, genotoxic, cytotoxic, and teratogenic. We postulate that the toxic mechanism of the mono, di, and tri HAAs is based on the inhibition of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a target glycolytic enzyme. The inhibition of GAPDH will lead to severe reduction in ATP levels due to the loss of pyruvate, which will lead to the mitochondrial stress and genomic DNA damage. We found that monoHAAs are the strongest GAPDH inhibitors in Chinese hamster ovary (CHO) treated cells and induced a highest rate of reduction in ATP levels as compared to the di and triHAAs. Where triHAAs were the weakest GAPDH inhibitors and showed an increase in the ATP levels in CHO treated cells as compared to the negative control. Since triHAAs did not reduce the ATP levels unlike monoHAAs so these data suggest that the triHAAs have a different target molecule for its toxicity. Based on the previous literature and our current data, we recommend to study the impact of di and triHAAs on the pyruvate dehydrogdenase activity, which is responsible for the introduction of pyruvate to mitochondria, fueling the Kreb cycle and production of ATP. The results from this study support the hypothesis that GAPDH inhibition and subsequent generation of reactive oxygen species is linked with the toxicity mechanism of monoHAAs and disturbance in the cellular glucose and ATP homeostasis is responsible for the toxicity induced by di and triHAAs.

2

Characterization of BaP-Induced lacZ Mutations in the Bone Marrow and Sperm of Muta™Mouse Males Using Next-Generation Sequencing. Beal MA[1], Gagne R[2], O'Brien JM[2], Yauk CL[2], Marchetti F[2]. [1]Carleton University, Ottawa, ON, Canada, [2]Health Canada, Ottawa, ON, Canada.

The transgenic rodent gene mutation assay is a useful tool for quantifying induced gene mutations occurring *in vivo* in any tissue. The assay can be paired with sequencing to provide insight into the types of mutations occurring and degree of clonality. Until recently, DNA sequence analysis of long transgenes, such as lacZ (3096 bp), was laborious and expensive. As such, sequencing was traditionally performed on shorter transgenes, such as cII (294 bp). Here, we used next-generation sequencing (NGS) to simultaneously sequence ~5500 mutant lacZ transgenes from the bone marrow and sperm DNA of control Muta™Mouse males and males exposed to benzo[a]pyrene, an established somatic and germ cell mutagen. Sperm were collected 42 days after exposure to measure mutations induced in dividing spermatogonia. Using NGS we identified the locations and types of 3,599 lacZ mutations. The background mutation spectrum in sperm had a greater proportion of G:C to A:T transitions than bone marrow. BaP caused primarily guanine base substitutions in both tissues. However, BaP induced a higher proportion of G:C to C:G mutations in bone marrow and more deletions in sperm suggesting a difference in the repair of BaP-induced lesions in the two tissues. Clonality was higher in unexposed tissues, and thus correcting for clonality resulted in a higher measured mutagenic response in both tissues. These results demonstrate the utility of NGS in rapidly establishing the mutation spectra in multiple tissues for elucidating the mutagenic mode of action, comparing response across multiple tissues, and allowing for better assessment of genotoxic effects.

3

p53 May Be Important for Avoidance of Misleading Positives in *In Vitro* Genotoxicity Tests. Chapman KE, Seager AL, Brüsehafer K, Shah UK, Wilde E, Clatworthy ML, Mikhail JM, Johnson GE, Doak SH, Jenkins GJS. Swansea University, Swansea, West Glamorgan, United Kingdom.

Current *in vitro* genotoxicity tests display low sensitivity, as illustrated by the high frequency of misleading positive results. Therefore, identification of a biomarker that allows genotoxic agents to be distinguished from non-genotoxins could be advantageous. A possible candidate for such a biomarker is p53, a protein crucial in coordinating the DNA damage response. Here, the *In Vitro* Micronucleus Assay, Western blotting, mutation spectra, and comparison of human lymphoblastoid cell lines were used to establish whether p53 expression and status influenced genotoxicity. Protein analysis demonstrated that p53 was up-regulated in response to doses of hydrogen peroxide between 10μM and 20μM, coinciding with the LOGEL of 12μM for micronucleus frequency. In contrast, di(ethylhexyl)phthalate, which produced a negative micronucleus response, did not produce changes in p53 expression. Comparisons between p53-proficient cell line TK6, and a p53-deficient cell line isogenic to TK6, NH32, demonstrated that p53 could be responsible for an adaptive response following 0.009μg/ml *N*-methyl-*N*-nitrosourea. Further, greater micronucleus induction was observed in NH32 compared to TK6 following mitomycin-C or cytosine arabinoside treatments. Mutation spectra analysis confirmed that cell lines MCL-5 and AHH-1 both contain a partial CCGG to TTGG transition mutation at codon 282 in exon 8 of the *TP53* gene, which encodes p53. Such a mutation might confer heightened sensitivity to genotoxins, relative to cells with wild-type p53 status. Together, these data demonstrate that p53 is a promising candidate for distinguishing between genotoxins and non-genotoxins. Such findings might be useful for avoiding misleading *in vitro* genotoxicity positives, preventing unnecessary animal follow-up tests.

4

Computational Systems Biology Modeling of DNA-Damage Stress Pathways for Assessing Mutation Rates at Low Doses. Clewell RA[1], Pendse S[1], McMullen P[1], Sun B[1], Zhang Q[1], Adeleye Y[2], Carmichael P[2], Andersen ME[1]. [1]The Hamner Institutes for Health Sciences, Research Triangle Park, NC, United States, [2]Unilever, PLC, Sharnbrook, Bedfordshire, United Kingdom.

Homeostasis by cellular stress response pathways involves negative feedback acting through a series of steps. Many stress response pathways have rapid response, post-translational signaling and slower signaling through transcriptional upregulation. We examined multiple biological read-outs in a human cell line (HT1080) treated with several DNA-damaging compounds to support mechanistic computational modeling for micronuclei (MN) formation across wide dose ranges. The readouts included dose and time-dependent whole genome gene expression, DNA repair center (DRC) formation through high content imaging of pH2AX and p5BP1, as well as measures of key proteins in the p53 pathway and MN. Transcriptional upregulation only occurred at concentrations with clear increases in MN formation. Post-translational activation of DNA-repair processes acting through specific kinases appears to be the main contributor to regulation of DNA-damage at lower doses. We have developed computational pathway models to describe the relationship between DRCs and MN formation for two potent double strand break inducers with very different MN dose-response curves: etoposide (linear) and the gamma irradiation mimic neocarzinostatin (threshold-like). These models, ranging from simple empirical descriptions of the data to more biologically oriented descriptions of the homeostatic feedback loops, provide a quantitative framework for assessing the key processes governing MN prevention at low doses of different types of DNA damaging chemicals. Ultimately, these models will support decisions for *in vitro* only risk assessments by providing a quantitative description of how low dose threshold behavior may be achieved in mutation response, and helping define concentrations leading to cellular adaption and potential adversity.

5

Connecting Oxidative Damage to Epigenetic Alterations. Ding N[1], DeStefano Shields C[2], Sears CL[2], Baylin SB[2], O'Hagan HM[1]. [1]Indiana University, Bloomington, IN, United States, [2]Johns Hopkins University, Baltimore, MD, United States.

Inflammation and the associated increase in reactive oxygen species play a key role in the initiation and progression of a majority of human epithelial cancers. Many groups have demonstrated that inflammation causes alterations in DNA methylation, microRNA expression, and histone marks. Since by definition these epigenetic changes are mitotically heritable and affect gene expression, they likely play a role in establishing disease phenotypes. During carcinogenesis, aberrant gains in promoter DNA methylation transcriptionally silence tumor suppressor genes, linking DNA methylation directly to tumorigenesis. However, it is unknown what the mechanisms of targeting and initiation are for these stable disease-specific epigenetic marks. Previously, we have demonstrated that oxidative damage results in the relocalization of epigenetic silencing proteins from non-GC-rich to GC-rich areas of the genome, including CpG-islands of which some are DNA hypermethylated and silenced in cancer cells. This relocalization results in histone mark and nascent transcription changes. Our current research focuses on establishing the connection between DNA damage repair proteins and recruitment of these epigenetic silencing proteins to chromatin. Furthermore, using a mouse model of inflammation-induced tumorigenesis, we demonstrated that, in inflamed tissue, oxidative DNA damage and enrichment of epigenetic silencing proteins occurs in CpG island-containing promoters of key genes. We are currently using this model to study the molecular progression from acute oxidative damage-induced chromatin changes to permanent epigenetic silencing events during tumorigenesis. This work uniquely links oxidative damage to genome-wide changes in binding of epigenetic silencing proteins, describing a potential mechanism for the initiation of epigenetic changes in cancer.

6

Large Transcription Units Are Hotspots for Copy Number Variants Induced by Replication Stress. Glover TW, Arlt MF, Park SH, Rajendran S, Paulson M, Ljunjman M, Wilson TE. University of Michigan, Ann Arbor, MI, United States.

Copy number variants (CNVs) are a major factor in genomic structural variation, genetic disorders and cancer. However, there is limited understanding of how CNVs arise and the environmental and genetic risk factors involved. We previously demonstrated that agents that inhibit DNA replication, including aphidicolin, hydroxyurea and low-dose IR, induce a high frequency of CNVs in cultured human and mouse cells that mimic a large class of human CNVs. Working with these large sets of *de novo* CNVs, we show here that hotspots for these CNVs occur in large transcribed genes. Using Bru-seq nascent RNA sequencing, we demonstrated that the CNV hotpots specifically correspond to the largest transcription units in both human and mouse cells and that even genome regions with low frequencies of sporadic and induced CNVs are enriched in transcribed genes. Unlike most genes, these very large transcription units replicate late and organize deletion and duplication CNVs into their transcribed and flanking regions, respectively. We further demonstrated that these CNV hotspots coincide with common fragile sites demonstrating that these two distinct forms of structural alterations are different manifestations of replication inhibition at the same sensitive human loci. Consistently, transcription units >1 Mb are shown to be a robust and highly cell-type-specific predictor of CNV and CFS occurrence in a cells in which they are actively transcribed. These results establish that a dangerous combination of expansive transcription and late replication drives extreme local genomic instability under replication stress, with CNVs and CFSs representing different sequelae at the same cell-type-specific loci.

7

CD48-Deficient T-lymphocytes from ENU-Treated Rats Have Mutations in the *Pig-a* Gene. Dobrovolsky VN, Mittelstaedt RA, Pearce MG, Petibone DM, Revollo J. National Center for Toxicological Research, US FDA, Jefferson, AR, United States.

The *Pig-a* assay detects cells deficient in GPI-anchored protein surface markers by flow cytometry. Although the assumption is that the marker deficiency is due to mutations in the *Pig-a* gene, in theory, marker deficiency could be caused by events other than *Pig-a* mutation, e.g., gene silencing. In the present study, we investigated if deficiency in the GPI-anchored surface marker CD48 in rat T-cells is accompanied by mutation in the *Pig-a* gene. We treated male F344 rats with N-ethyl-N-nitrosourea and sorted CD48-deficient T-lymphocytes from their spleens with a flow cytometer into 96-well plates for clonal expansion. Sanger sequencing of individual clones confirmed that the expanded sorted cells have mutations in the *Pig-a* gene - primarily base-pair substitutions at A:T with the mutated T on the nontranscribed strand. This mutational pattern is consistent with the spectrum of ENU-induced mutation determined in other *in vivo* models. A similar mutational spectrum was determined in a pool of 64 expanded clones using analysis by next generation sequencing. These results indicate that *Pig-a* assays detect what they are designed to detect - gene mutation in the *Pig-a* gene.

Basic Platform Session 1

8

A Yeast Bioassay to Uncover Environmental Mediators of Germ Cell Copy Number Mutagenesis. Argueso JL, Chapman M, Stewart G, Maranon D. Colorado State University, Fort Collins, CO, United States.

De novo gene copy number variation (CNV) is now recognized as a significant source for a wide range of human diseases, including autism spectrum disorders. However, the environmental factors that may contribute to copy number mutagenesis are poorly understood. In the human germline, meiotic recurrent CNVs form through unequal crossing-over between low copy repeat elements (LCRs). Such large repeated sequences are found at CNV hotspot regions of our genome, but are absent in yeast. We created a yeast bioassay for meiotic CNVs by modifying the yeast genome through the introduction of engineered LCRs: segments of yeast or human DNA that we duplicated and integrated at specific chromosomes. The engineered LCRs flank allelic insertions of drug resistance and fluorescent protein markers whose segregation in the haploid cell progeny ("gametes") can be followed by tetrad analysis, fluorescence microscopy, and flow cytometry. Normal allelic recombination produces cells that express either marker by itself, but never both together. In contrast, haploid cells expressing both allelic markers simultaneously contain recurrent segmental duplications mediated by the engineered LCRs. Thus, the frequency of double fluorescent or double drug resistant cells is primarily a function of the rate of *de novo* meiotic CNV. We are developing this system to investigate meiotic CNV mechanisms and to interrogate the CNV stimulation activity of a diverse panel of candidate environmental copy number mutagens. This approach should help uncover environmental contaminants that may be increasing CNV formation in the germline of exposed human populations.

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Tissue-Specific Transcriptomic Analysis Undermines the Utility of Benzo(*a*)pyrene As a Point of Reference for Carcinogenic Polycyclic Aromatic Hydrocarbons. Labib S[1], Williams A[2], Guo CH[1], Arlt VM[3], Schmeiser HH[4], Leingartner K[2], Yauk CL[2], White PA[2,1], Halappanavar S[2,1]. [1]University of Ottawa, Ottawa, ON, Canada, [2]Health Canada, Ottawa, ON, Canada, [3]King's College London, London, United Kingdom, [4]German Cancer Research Center, Heidelberg, Germany.

Benzo(*a*)pyrene (BaP) is an index chemical for calculation of excess lifetime cancer risk (ELCR) of environmental chemical mixtures containing polycyclic aromatic hydrocarbons (PAH). This is based on the additivity assumption, which suggests that PAHs elicit carcinogenesis via similar genotoxic modes-of-action, which may be inaccurate. We analyzed global tissue-specific gene expression profiles elicited by eight PAHs routinely used in ELCR calculations of PAH mixtures to evaluate the accuracy of this assumption. Male Muta™Mouse were gavaged with three doses of eight PAHs for 28 days. Tissues were collected 3 days post-exposure. Whole-genome microarrays were used to profile hepatic, forestomach, and pulmonary tissue mRNA expression. Cancer-related biological processes perturbed in response to each PAH were identified using bioinformatics tools. Pulmonary EROD activity, DNA adducts, and lacZ mutant frequencies were measured to confirm metabolism and genotoxicity. The lungs showed a robust response across all PAHs. Therefore, pulmonary response will be the focus of the presentation. All PAHs induced pulmonary phase-1 enzyme activity and DNA adducts/mutation. Altered regulation of DNA damage response, immune/inflammatory response, and cell signalling processes was observed in response BaP and other PAHs. However, some pathways related to cancer promotion (e.g., calcium signaling and complement activation) were uniquely altered by PAHs other than BaP. Moreover, responses induced by benzo(*ghi*)perylene were not related to any known carcinogenic processes, suggesting different cancer mechanisms for each PAH. The PAHs' differential ability to perturb biological pathways at the molecular level challenges the additivity assumption, which may under/overestimate the cancer potential of PAH-rich environmental mixtures.

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Kinetic Study of Chromosomal Double-Strand Breaks with Diverse Break Structures Using High-Resolution Techniques. Liang Z, Nallasivam S, Wilson TE. University of Michigan, Ann Arbor, MI, United States.

Nonhomologous end joining (NHEJ) is the dominant double-strand break (DSB) repair pathway in cells with limited or no 5' resection. DSBs often harbor diverse break structures that can complicate rejoining and lead to mutations. To better understand how overhang polarity affects repair, we engineered an efficient system to induce site-specific 5'-overhanging DSBs (5' DSBs) in the *S. cerevisiae* genome using zinc finger nucleases (ZFNs). Improved activity of our ZFN system allows us to study for the first time the repair kinetics of 5' DSBs by chromatin immunoprecipitation and next-generation sequencing. Surprisingly, NHEJ factors, including Yku80, Pol4 and Dnl4, showed significantly higher recruitment to ZFN-induced 5' DSBs as compared to HO-induced 3' DSBs in the same locus. Consistently, NHEJ efficiency was higher at ZFN-induced 5' DSBs. We hypothesize that 3' and 5' DSBs may be processed differently in the break ends affecting the stability and/or activity of NHEJ. Kinetics of end-processing was analyzed using our developed ligation-mediated qPCR at single-nucleotide resolution. In addition, we demonstrated that yeast Tyrosyl-DNA phosphodiesterase 1 (Tdp1) was recruited at a low level exclusively to 5' DSBs and that its recruitment was antagonized by Ku. Conversely, overexpression of Tdp1 weakly compromised NHEJ. These findings suggest that Tdp1 competes with NHEJ at 5' DSBs. Moreover, sequencing of chromosomal 5'-DSB joints has not to date revealed evidence for Tdp1-mediated suppression of insertional mutagenesis as observed in plasmid studies. In summary, our study provides new insights of how overhang polarity at genomic DSBs influences end-processing and the course of mutagenesis.

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Using High-Throughput Assays to Measure Interindividual Differences in DNA Repair Capacity and to Understand Mechanisms of Cancer Therapy Resistance. Nagel ZD, Margulies CM, Chaim IA, McRee SK, Mazzucato P, Ahmad A, Abo RP, Butty VL, Forget AL, Samson LD. Massachusetts Institute of Technology, Cambridge, MA, United States.

The capacity to repair different types of DNA damage, relentlessly induced by endogenous and exogenous environmental agents, varies among individuals making them more or less susceptible to the detrimental health consequences of such exposures. Current methods for measuring DNA repair capacity (DRC) are relatively labor intensive, often indirect and usually limited to a single repair pathway. We present a fluorescence-based multiplex flow-cytometric host cell reactivation assay (FM-HCR) that measures the ability of cells to repair plasmid reporters each bearing a different type of DNA damage or different doses of the same type of DNA damage. FM-HCR can measure simultaneously repair capacity in up to four distinct pathways. We use FM-HCR to measure DRC in a panel of lymphoblastoid cell lines from apparently healthy individuals, and we show that the sensitivity of these cells to DNA damaging agents correlates with their DRC. This correlation suggests that FM-HCR could be applied to make predictions about the sensitivity of cancer cells to therapeutic DNA damaging agents. Interindividual differences in DRC have also been measured in peripheral blood mononuclear cells isolated from human donors. A next generation sequencing-based HCR assay (HCR-Seq) capable of higher-throughput DRC measurements was also developed. This assay detects rare transcriptional mutagenesis events due to lesion bypass by RNA polymerase, providing an added dimension to DRC measurements. Together, FM-HCR and HCR-Seq provide powerful next generation tools for measuring global DRC and exploring relationships among DRC, disease susceptibility, and optimal treatment.

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Aurora B Mediated Novel Function of DNA Double-Strand Break Response Protein 53BP1 *In Mitosis*. Wang H[1,2], Peng B[3], Engler DA[4], Xu X[3], Xu B[5], Mitra S[1], Hegde ML[1,2]. [1]Department of Radiation and Oncology, The Houston Methodist Hospital Research Institute, Houston, TX, United States, [2]Neurological Institute, The Methodist Hospital, Houston, TX, United States, [3]Beijing Key Laboratory of DNA Damage Response, Beijing, Beijing, China, [4]Proteomics Core, The Houston Methodist Hospital Research Institute, Houston, TX, United States, [5]Department of Biochemistry and Molecular Biology, Southern Research Institute, Birmingham, AL, United States.

In response to DNA double-strand breaks (DSBs), 53BP1 is recruited to the DSB sites for its damage repair function and cell cycle checkpoints regulation. However, during mitosis, instead of binding with DNA damage termini, 53BP1 specifically localizes on outer kinetochores and is hyperphosphorylated. It has been reported that mouse 53BP1$^{-/-}$ cells show high spontaneous chromosomal abnormalities compared with wild type cells, however, its mitotic role has not been investigated. We hypothesized that 53BP1 deficiency/functional defects may have tumorigenic implications in mouse or human organs by inducing mitotic defects. By using proteomic, molecular and cell biology methods including Mass Spectrometry, Immunofluorescence, Time Lapse Assa and so on, we have identified a novel phosphorylation site in 53BP1 that lies in the consensus motif of mitotic kinase Aurora B. We subsequently demonstrated that Aurora B phosphorylates 53BP1 *in vitro* and this phosphorylation is required for 53BP1's recruitment to kinetochores in U2OS cells. We further observed that 53BP1 is stabilized on merotelic kinetochore attachments (an error spontaneously occurs in the attachment of microtubule and kinetochore in mitosis has been considered as the major cause of aneuploidy) by mitotic centromere-associated kinesin (MCAK). Further, we found that depletion of 53BP1 significantly induces the lagging chromosome formation and difficulties of chromosome bridge abscission. The present study provides a molecular mechanism of the mitotic functions of 53BP1, and highlights the novel functions of 53BP1 in cell division by protecting against chromosome instability, which may have implications in carcinogenesis as well as the development of 53BP1 targeted tumor treatment.

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Uncovering New Genes, Proteins, and Pathways Regulated by the ARTD1/PARG Axis. Fouquerel E, Yu Z, Li J, Sobol RW. University of Pittsburgh, Pittsburgh, PA, United States.

The human genome encodes 17 poly(ADP-ribose) polymerase (PARP) or ADP-ribosyltransferase diphtheria toxin-like (ARTD) proteins that are involved in regulating a variety of cellular processes. These include, DNA damage signaling and repair, chromatin remodeling, transcription, epigenetic gene regulation, mitosis and differentiation. ARTD1 (PARP1) is a key enzyme involved in DNA repair by synthesizing poly(ADP-ribose) (PAR) in response to strand breaks. Conversely, PARG functions to attenuate the biological impact of PAR by degrading and hydrolyzing the PAR polymer. Together, ARTD1 and PARG provide a counter-weight to effectively regulate the global PAR response. We established a proteomics based PAR-interactome after DNA damage that reveals new targets of ARTD1/PARG regulation. In addition to regulating cellular function via post-translational modification, ARTD1 and PARG directly regulate the expression of select genes. We therefore developed and transcriptionally analyzed isogenic ARTD1 and PARG depleted cells to uncover genes regulated by the ARTD1/PARG regulatory pathway. In all, these studies have revealed new genes, proteins and pathways regulated by the ARTD1/PARG axis that may be considered environmentally sensitive genes depending on the cell type and genotoxic stimuli.

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Effect of Intestinal Microbiota on Lymphoma and Longevity in Atm Deficient Mice. Schiestl R, Liu J. University of California, Los Angeles, Los Angeles, CA, United States.

Intestinal microbiota plays a role in the nutrient metabolism, modulation of the immune system, arthritis, obesity and intestinal inflammation. Our move from Harvard to UCLA led to a huge decrease in genetic instability and logevity in Atm deficient mice. A change of the intestinal microbiota to conventional microbiota led to the same phenotype at Harvard. We tested Atm deficient mice for genotoxicity, DNA damage, inflammation markers, cancer latency and longevity and high throughput sequencing of the intestinal microbiota. Isogenic mice from different housing facilities showed a fourfold difference in life expectancy, a 4.5 fold difference in genetic instability and DNA damage. The onset of lymphomas was significantly 2 fold different. We sequenced the microbiota of both facilities and found Lactobacillus johnsonii 456 as dominant bacterial strain in the health beneficial microbiota. Just this bacterium by itself reduced genotoxicity, reduced inflammation, and reduced levels of cytotoxic T cells in the liver and blood. We also found similar differences in Trp53 deficient and even in wildtype mice. Thus, we have shown that the intestinal microbiota is responsible for differences in genetic instability, genotoxicity, DNA damage, inflammation, latency of lymphoma and longevity. The underlying mechanisms is probably due to inflammation promotion or suppression mediated by the intestinal microbiota. The understanding of this effect may lead to a breakthrough in the understanding of the causes of carcinogenesis, which might lead to prevention of AT, a currently incurable progressive disease and possibly other cancer-prone DNA repair deficient diseases or even wildtype mice and people.

Applied Platform Session 2

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Identification of Single Nucleotide Polymorphisms and Novel Genetic Anomalies in the Normalized Transcriptomes of TK6, WTK1, and NH32 Cells by Next Generation Sequencing. Revollo J, Petibone D, Morris SM, Ning B, Dobrovolsky V. National Center for Toxicological Research, Jefferson, AR, United States.

TK6, WTK1, and NH32 cells are human lymphoblastoid cell lines with a common origin. Since they are heterozygous at the thymidine kinase locus (TK+/-), they are routinely used to assess the mutagenicity of chemicals by using loss of heterozygosity of the remaining wild-type TK allele as a reporter. Despite their common use and importance in genetic toxicology, little is known about other genetic abnormalities these cells may possess and how these may affect their response to genotoxicants. To catalog mutations carried by TK6, WTK1, and NH32 cells, we analyzed their normalized transcriptomes by 454 Sequencing. We detected thousands of genetic variations arising from nuclear and mitochondrial DNA, the vast majority of which were commonly shared by TK6, WTK1, and NH32. Importantly, we detected several anomalies that could affect the genotoxic profile of these cells, such as clinically-relevant SNPs at the xenobiotic metabolizing enzyme NADPH quinine oxidoreductase 1 (NQO1) and the thiopurine drugs metabolizing enzyme thiopurine S-methyltransferase (TPMT) loci. Our data provide a comprehensive catalog of genetic anomalies in the transcriptomes of TK6, WTK1, and NH32.

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Characterization of Primary Muta™Mouse Hepatocytes: A Promising New *In Vitro* Tool for Mutagenicity Assessment. Cox JA[1], Zwart EP[2], Luijten M[2], White PA[1,3]. [1]Department of Biology, University of Ottawa, Ottawa, ON, Canada, [2]Laboratory for Health Protection Research, National Institute for Public Health and the Environment, Bilthoven, Utrecht, Netherlands, [3]Mechanistic Studies Division, Environmental Health Science and Research Bureau, HECSB, Health Canada, Ottawa, ON, Canada.

The field of genetic toxicology is moving away from large-scale *in vivo* studies towards predictive *in vitro* methods. This shift, as well as deficiencies associated with the current *in vitro* genotoxicity test battery, has revealed the necessity for novel *in vitro* assays. Primary hepatocytes from the Muta™Mouse, a transgenic rodent, are ideal for *in vitro* assays due to their inherent metabolic capacity, stable karyotype, and well-validated mutation scoring system. A primary hepatocyte isolation protocol was optimized to maximize viable cell yield. The isolated cells were found to have a hepatocyte-like morphology, with a large proportion of binucleated cells. Fluorescent immunocytochemistry revealed that the isolated cell population expressed hepatocyte-specific markers, such as albumin and cytokeratin 18. The basal ethoxyresorufin O-deethylase (EROD) activity, a measure of cytochrome P450 1A1 and 1A2, maintained a level of 9.6 ± 1.2 pmol resorufin/mg protein/min over time in culture, whereas benzo[a]pyrene (BaP) induced EROD activity increased in culture to a maximal 139.0 ± 8.5 pmol resorufin/mg protein/min three days post isolation. Glutathione-S-transferase (GST) activity increased more than 2-fold over three days in culture, but uridine 5'diphospho-glucuronosyltransferase (UDPGT) activity remained constant at 1.9 nmol 4-methylumbelliferone/mg protein/min. Mutant frequency fold-changes of 11.4 and 4.9 over control were observed for known mutagens, BaP and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), respectively. Metabolic enzyme gene expression analysis, karyotype analysis, and further validation are ongoing. With their metabolic competence and convenient transgenic mutation targets, primary hepatocytes from the Muta™Mouse are an innovative solution to the problems posed by existing *in vitro* mammalian cell assays.

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CometChip: Enabling Translation of DNA Damage and Repair Assays. Ge J, Tay JJI, Su Y, Mazzucato P, Chow DN, Fessler J, Weingeist DM, Wood DM, Engelward BP. Massachusetts Institute of Technology, Cambridge, MA, United States.

DNA damage promotes cancer, aging, neurological disorders, and heritable diseases. Exposure to DNA damage is unavoidable, as DNA damaging agents are ubiquitous both in our environment and within our cells. We have previously reported the development of "CometChip", a novel technology for measuring DNA damage that has its basis in the well-accepted Comet assay. Recently, we have established methods for applying the device to studies of DNA repair capacity in people. Using repair deficient cell lines, we demonstrated the efficacy of the approach for identifying cells that harbor defects in four major repair pathways: base excision repair, nucleotide excision repair, mismatch repair, and nonhomologous end-joining. We have demonstrated the efficacy of the platform for high throughput screening presence of defects in these pathways and screened cells from ethnically diverse individuals to reveal inter-individual variation in DNA repair. Finally, we have applied this new CometChip repair assay to study multi-pathway roles of two genes: Xrcc1 and DNA-PKcs, revealing novel functions of these two genes. Taken together, we have both learned about inter-individual differences and we have broadened our knowledge about repair functions of key repair proteins.

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MicroRNA-mRNA Regulatory Networks Help to Unravel Mechanisms of Response to BaP Exposure. Lizarraga D[1,2], Gaj S[1], Brauers KJ[1], Timmermans L[1], Kleinjans JC[1], van Delft JH[1]. [1]Netherlands Toxicogenomics Centre, Maastricht University, Maastricht, Netherlands, [2]School of Public Health, University of California, Berkeley, Berkeley, CA, United States.

MicroRNAs have emerged as attractive candidate biomolecules to explore the impact of environmental exposures on health outcomes, due to their role in cellular processes and inherent stability. In this study we have identified microRNA-mRNA networks as novel mechanisms of response to Benzo[a]pyrene (BaP) exposure. BaP, a polycyclic aromatic hydrocarbon environmental pollutant, was used as a model genotoxic/carcinogenic compound. We evaluated time-dependent effects of 2 μM BaP on mRNA and microRNA profiles in HepG2 cells, a widely used human liver cell line that expresses active p53, critical for genotoxic responses to BaP. Significant changes in expression of multiple microRNAs and downstream target genes were observed. A pathway analysis approach was used to evaluate the relevance of these deregulated microRNAs to genotoxicity. Eight microRNAs were involved in important genotoxicity-related pathways such as apoptotic signaling, cell cycle arrest, DNA damage response and repair. This study highlights the potential role of microRNA-29b, microRNA-26a-1*, and microRNA-122* as novel players in the BaP response. This study demonstrates the added value of an integrated microRNA-mRNA network approach for identifying molecular mechanisms involved in response to environmental pollutants such as BaP in an *in vitro* human model.

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Characterization of microRNAs in Serum: A New Class of Biomarkers for Diagnosis of Parkinson Disease in Mouse Model. Chigurupati S, Raymick J, Paule MG, Sarkar S. National Center for Toxicological Research, US FDA, Jefferson, AR, United States.

Parkinson disease (PD) is the second most common neurodegenerative disease with unknown etiology. No available therapies change the underlying neurodegenerative process. However, symptomatic therapies can improve patient quality of life to some extent. The accuracy of the clinical diagnosis of PD is very limited, especially in the early stages, when cardinal symptoms are not conclusive. The current study was conducted to find an early and non-invasive biomarker in serum samples of mouse MPTP (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine) PD model. Dysregulated expression of microRNAs (miRNAs) in various tissues has been associated with a variety of diseases, including neurodegenerative diseases. Here we demonstrate that miRNAs are present in the serum and plasma of MPTP mouse model. The levels of miRNAs in serum are reproducible, consistent and stable. Employing 454 sequencing, relatively new technology, we analyzed the miRNA serum composition of control and a PD mouse model. We identified specific expression patterns of serum miRNAs in healthy versus PD mouse models. Through these analyses, we conclude that serum miRNAs serve as potential biomarkers for the early detection of PD.

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Multiplexed, High Information Content DNA-Damage Response Assay Discriminates Clastogens, Aneugens, and Cytotoxicants. Bryce SM, Bemis JC, Carlson KM, Berg AL, Dertinger SD. Litron Laboratories, Rochester, NY, United States.

This laboratory has previously evaluated candidate biomarkers associated with DNA damage response pathways for their ability to discriminate *in vitro* clastogens, aneugens, and nongenotoxic cytotoxicants. The objective of the current work was to multiplex promising endpoints into a single "add-and-read" type assay that could be analyzed via high throughput flow cytometry. TK6 cells were exposed to 7 clastogens, 7 aneugens, or 3 cytotoxicants in 96 well plates over a range of concentrations. At 4 and 24 hrs, 25 microliters were removed from each well and added to a 384 well plate containing a reagent mix consisting of a detergent to liberate nuclei, propidium iodide and RNase to serve as a pan-DNA dye, fluorescent antibodies against gH2AX, cleaved PARP, and phospho-H3, and known concentration of Counting Beads for absolute cell (nuclei) counts. Robotic sampling allowed flow cytometric data acquisition to occur in a walk away mode of operation. To simplify data evaluation, a normalization strategy was employed whereby data associated with one equitoxic concentration was considered (approximately 25% relative nuclei count at 24 hrs). Using this strategy, a logistic regression model identified 4 hr gH2AX shift, 4 hr fold-increase in %phospho-H3-positive events, and 24 hr fold-increase in polyploidy cells as predictive factors. Furthermore, the three-factor model grouped each of the 18 chemicals into the correct *a priori* classification. These data support the hypothesis that a matrix of high throughput-compatible biomarkers can effectively delineate two important modes of genotoxic action as well as identify cytotoxicity that can lead to irrelevant positive results.

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Multi-Endpoint Comparison of Low-Dose Responses to Benzo(*a*)pyrene. Long AS[1], Arlt VM[2], Dertinger SD[3], White PA[1,4]. [1]Department of Biology, Faculty of Graduate and Postdoctoral Studies, University of Ottawa, Ottawa, ON, Canada, [2]Analytical and Environmental Sciences Division, MRC-HPA Centre for Environment and Health, King's College London, London, United Kingdom, [3]Litron Laboratories, Rochester, NY, United States, [4]Mechanistic Studies Division, Environmental Health Science and Research Bureau, Health Canada, Ottawa, ON, Canada.

Regulatory evaluation of genotoxic carcinogens commonly assumes that the dose-response is linear (i.e., no dose below which the effect is negligible). This assumption has been questioned; however, the vast majority of existing data relate to effects observed only at high doses, and determination of a point-of-departure (PoD) is rarely possible. Using benzo(*a*)pyrene (BaP) as a model genotoxic carcinogen, we have examined the dose-response relationships for four different endpoints, in multiple tissues, to scrutinize the assumption of low-dose linearity. Adult male Muta™Mouse specimens were exposed sub-chronically to 11 doses of BaP (ranging from 0.1-50 mg/kg/day) via oral gavage. The frequency of stable DNA adducts and *lacZ* mutants were measured in bone marrow, and the frequency of *Pig-a* mutations and MN (micronuclei) were assessed in reticulocytes (RETs) and red blood cells (RBCs or NCEs). Various types of mathematical models were fit to each data set, and, with the exception of MN induction, the best model for all dose-responses was non-linear. Benchmark Dose (i.e., $BMDL_{10}$) values were calculated for each endpoint. The lowest $BMDL_{10}$ was obtained for DNA adducts (0.35 mg/kg/day), followed by transgene mutations (1.20), MN-NCE (3.34), Pig-a-RBC (3.67), and finally MN-RET (3.88). The observed PoD pattern (e.g., lowest for adducts) is consistent with the sequence of key events leading to mutations and/or cytogenetic damage. The observation of non-linear responses is consistent with the existence of compensatory mechanisms (i.e., DNA repair) that are effective at low doses. The frequency of adducts and transgene mutations are currently being assessed in other tissues.

Basic Platform Session 2

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Impact of Repetitive Element Transcriptional Activation in Cocaine Addiction. Wang T[1], Feng J[2], Fargo D[1], Santos J[1], Nestler E[2], Woychik R[1]. [1]National Institute of Environmental Health Sciences, Durham, NC, United States, [2]Mount Sinai Medical School, New York, NY, United States.

Expression of repetitive elements (REs), which comprise about 50% of the mammalian genome, can impact DNA stability through transposition and reintegration. REs may also interfere with transcription of genes located in their immediate vicinity. We have shown that normal regulation of the *agouti* gene in mice can be hijacked by an intracisternal A particle (IAP) inserted within the first intron of the gene. The result is a chimeric RNA transcript containing the IAP sequence followed by the normal agouti sequences. Such ability of the IAP to control *agouti* expression is dependent upon the epigenetic status of the IAP: methylation of this element effectively silences its expression and its genetic control of *agouti.* We hypothesized that RE cis-regulation of downstream genes is a common mechanism associated with gene expression responses to environmental stimuli. To test this, we developed a bioinformatics framework to study the expression of all classes of REs and to find fusion transcripts between an individual RE and a closely linked non-repetitive gene along the chromosome. We used RNA-seq data sets derived from the nucleus accumbens (NA) of cocaine-treated and control mice since repeated cocaine exposure leads to the de-repression of several REs. Our results show that ~805K RE loci are expressed in the NA, from which 2,500 are differentially regulated by cocaine. Notably, we found chimeric reads between a RE and an adjacent non-RE sequence in 490 genes. This research was supported in part by the Intramural Research Program of the NIH, National Institute of Environmental Health Sciences.

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Topoisomerase IIa Gene Promoter Methylation Is Higher and Correlates with Cumulative Time of Exposure in Workers Exposed to a Volatile Organic Compound Mixture including Benzene. Jiménez-Garza O[1], Baccarelli A[2], Byun H-M[2], Guo L[3], Zhong J[2]. [1]University of Guanajuato, León Campus, Health Sciences Division, León, Guanajuato, Mexico, [2]Harvard School of Public Health, Laboratory of Human Environmental Epigenomics, Boston, MA, United States, [3]Key Laboratory of Pollution Processes and Environmental Criteria, College of Environmental Sciences and Engineering, Nankai University, Tianjin, China.

Occupational exposure to high levels of benzene (BZ) has shown a decreased expression of Topoisomerase IIa (TOP2A) in bone marrow mononuclear cells; however, DNA promoter methylation for this exposure has not been investigated so far. The goal of this work was to determine promoter methylation levels in genes related to DNA repair, inflammation and oxidative stress in workers exposed to a volatile organic compound (VOC) mixture including benzene. Workers from three different labor scenarios exposed to VOCs were included, as well as a reference group. According to the individual exposure levels to BZ, we classified the exposed groups in null exposure (plastic shoes factory, PS), very low exposure (leather shoes factory, LS) and low exposure (gas station attendants, GS). We took peripheral blood samples for DNA extraction and bisulfite treatment. After primer design, we performed PCR-Pyrosequencing in order to measure methylation levels in 13 different gene promoters. LS workers exposure was higher for four VOCs other than BZ. This group showed hypermethylation for TOP2A, TNFa and SOD1 promoter genes. Hypermethylation for the IL6 gene was observed in GS workers. TOP2A Methylation levels negatively correlated with cumulative time of exposure and BZ exposure levels in LS workers. Also, TOP2A methylation levels correlated differentially with methyl acetate exposure levels in two different labor scenarios. In spite of a lower BZ exposure, the high TOP2A promoter Methylation and its corresponding correlations observed in LS workers may be attributable to a synergistic effect caused by the concurrent exposure to other, possibly carcinogenic VOCs.

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Short- and Long-Term Epigenetic Effects of Exposure to Six Sources of Particulate Matter. Miousse IR, Chalbot MCG, Nzabarushimana E, Kavouras IG, Koturbash I. University of Arkansas for Medical Sciences, Little Rock, AR, United States.

Particulate matter (PM), a major component of outdoor air pollution, has recently been classified as a human lung carcinogen by the International Agency for Research on Cancer. Previous studies performed in our laboratory demonstrated that ambient particulate matter induced epigenetic alterations in mouse macrophages after 24h *in vitro* exposure. The objective of the current study was to examine the effects of particle types on the cellular epigenome. To achieve this, we determined the levels of repetitive elements-associated DNA methylation and expression of DNA methylation machinery genes in response to exposure to particles from six different sources: traffic, wood burning, road dust, agricultural dust, natural dust, and pollen. Epigenetic responses were assessed in murine RAW264.7 macrophages and human primary epithelial airway cells (SAEC) after exposure to PM concentrations of 5 and 50 µg/mL, and at two time points: 24h and 72h of exposure. The chemical composition of atmospheric aerosol was determined by NMR, ICP/MS isotopic ^{13}C, and TOR analysis. We show that PM from various sources has different abilities to generate an epigenetic response. Particularly, PM extracted from natural, road, and agricultural dust was capable of inducing a rapid decline in DNA methyltransferases expression. This was followed by the hypomethylation of repetitive elements after 72h of exposure to PM from natural and agricultural dust as well as traffic-derived PM. Our data suggest that the extent of the epigenetic response was dependent on the source of PM.

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Evaluating Genomic Damages and *GSTM1* and *GSTT1* Polymorphisms in Rural Workers Occupationally Exposed to Pesticides: A Case-Control Study in an Agropastoral Brazilian State. Silva DM[1,2], Carvalho WF[2], Melo COA[1,3], Godoy FR[4], Bastos RP[4], Cruz AD[3,4], Franco FC[1], Arruda AA[1]. [1]Universidade Federal de Goiás, Programa de Pós-Graduação em Genética e Biologia Molecular, Goiânia, Brazil, [2]Universidade Federal de Goiás, Programa de Pós-Graduação em Ciências Ambientais, Goiânia, Brazil, [3]Pontifícia Universidade Católica de Goiás, Mestrado em Genética, Goiânia, Brazil, [4]Universidade Federal de Goiás, Programa de Pós-Graduação em Biotecnologia e Biodiversidade, Goiânia, Brazil.

We analyzed 139 individuals, consisting of 71 individuals occupationally exposed to pesticides and 68 of the control group, which had ethnic and socioenvironmental similarities. To evaluate mutagenic and genomic potential of pesticides we collected oral cavity cells and whole blood samples for micronucleus test and comet assay, respectively. Whole blood samples was also evaluated to analyze the *GSTT1* and *GSTM1* polymorphisms by real time PCR (qPCR), showing the specific melting curves for both loci. We observed a statistically significant difference between the exposed and control (p<0.001) groups for comet assay's parameters, frequency of micronuclei and binucleated cells. Thus, our results demonstrated that, both genomic damages and micronuclei frequencies are directly related to occupational exposure to pesticides. The frequency distribution of *GSTM1* and *GSTT1* null genotypes in the exposed group was observed in 43.66% and 12.21%, respectively. The control group showed deletion of *GSTM1* in 39.70% of subjects, and in the *GSTT1*, 29.41% of patients presented a deletion; however, there was an increased risk of intoxication for the null genotypes. Individuals exposed to pesticides that presented the *GSTT1* null genotype had the highest values of the three comet assay parameters, as the highest micronucleus and binucleated frequencies. So, the genetic monitoring should be considered as part of good medical supervision in people in direct contact with pesticides, since it allows evaluating the potential risk of occupational exposure, making possible the implementation of measures for the early identification of genetic risk.

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Putative Early-Life Epigenetic Biomarkers of Hepatocellular Carcinoma in Mice Perinatally Exposed to Bisphenol A. Weinhouse C, Nahar MS, Anderson OS, Dolinoy DC. University of Michigan School of Public Health, Ann Arbor, MI, United States.

Bisphenol A (BPA) is an endocrine disrupting chemical (EDC) that has been implicated as a potential carcinogen and epigenotoxicant. We have previously reported dose-dependent incidence of hepatic tumors in 10-month-old isogenic mice perinatally exposed to BPA through maternal diet (50 ng BPA/kg diet, 50 µg BPA/kg diet, 50 mg BPA/kg diet or a control diet). In order to investigate potential developmental origins of this observation, we evaluated DNA methylation via bisulfite sequencing at three candidate genes (*Esr1*, *Il-6st*, and *Stat3*) in liver tissue of BPA-exposed mice euthanized at two timepoints: 10 months of age (n=78, including n=18 with hepatic tumors) or post-natal day 22 (PND22; n=147). Candidate genes were chosen based on known expression changes in both rodent and human hepatocellular carcinoma. 10-month tumor presence was significantly associated with 6% lower mean methylation at *Stat3* (p<0.0037) and showed interaction with dose in 50 µg BPA and 50 mg BPA groups (p=0.0089 and p=0.0803, respectively), as compared to control. Dose was associated with 2% lower mean methylation at *Stat3* in PND22 mice (ANOVA p=0.0095), specifically in 50 µg and 50 mg BPA groups (p=0.0017 and p=0.0068, respectively.) BPA exposure but not tumor presence was marginally associated with higher mean methylation at *Esr1* and *Il-6st* at both ages. These data implicate *Stat3* as a potential early life biomarker of adult murine liver tumor risk following early BPA exposure. The characterization of environmentally influenced biomarkers of disease is a crucial next step for the development of novel screening targets for human disease prevention.

27

PON1 As a Model for Integration of Genetic, Epigenetic, and Expression Data on Candidate Susceptibility Genes. Huen Northcote K, Yousefi P, Thomas R, Eskenazi B, Holland N. University of California, Berkeley, Berkeley, CA, United States.

Epigenetics plays an important mechanistic role in disease development and is a likely interface through which environmental exposures affect gene expression and adverse health. Here, we used Paraoxonase 1 (PON1) as a model for integrating genetic, epigenetic, and expression data on a candidate susceptibility gene. PON1 is a multifunctional enzyme involved in oxidant defense and organophosphate (OP) pesticide detoxification. We found a broad variability of PON1 levels and substrate-specific activities in Mexican-American children from the Center for Health Assessment of Mothers and Children from Salinas (CHAMACOS) study. Susceptible PON1 genotypes and low PON1 quantity were associated with poorer mental and psychomotor development at age 2, and lower IQ at age 7. Genetics and age are important determinants of PON1 levels but do not completely explain the broad variability of PON1 molecular phenotype. Epigenetics of PON1 has been largely unexplored. In this study, we assessed DNA methylation in 18 CpG sites located along PON1 shores, shelves, and islands in newborns and 9-year-old children (Illumina 450K Methylation BeadChip) and observed significant decreases in arylesterase activity (protein levels) and mRNA expression (qPCR) with increased methylation at 8 CpG sites (p-values: 0.02-0.0002). PON1-108 genotype was strongly associated with DNA methylation particularly among sites in the CpG island (p<<0.0005). We also identified a relationship between expression of circulating miRNAs with putative PON1 binding sites (Affymetrix miRNA 4.0 array) and PON1 arylesterase activity. Our data suggest both genetics and epigenetics contribute to PON1 variability and thus potential susceptibility to adverse neurodevelopment outcomes.

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Overlapping and Distinct Effects of Bisphenol A and Its Substitute BPS on Germ Cells. Chen Y, Allard P. Molecular Toxicology Interdepartmental Program, University of California, Los Angeles, Los Angeles, CA, United States.

While the toxic effects of Bisphenol A (BPA) have come under heavy scrutiny, Bisphenol S (BPS), one substitute of BPA, is increasingly used in the "BPA-free" plastic manufacturing industry. Sharing a similar structure and bioactivity with BPA, BPS also poses a potential threat to human health. However, information regarding the toxicity of BPS is still limited. In this study, the reproductive toxicity of BPS is investigated by using the nematode *Caenohabditits elegans* (*C.elegans*). Worms were exposed to a range of concentration of BPA and BPS (125, 250 and 500µM) as well as a combination of both. We found that the worm's fertility decreases in a dose dependent fashion following exposure to either BPA or BPS. Surprisingly, co-exposure shows synergistic effects between BPA and BPS and a sharp decline in the fertility of the worms. Further analysis revealed that the decreased fertility observed with BPA or BPS is due in part to an increased embryonic lethality as well as an increase in germline apoptosis levels. We also showed that these high levels of embryonic lethality are likely to originate from the fertilization of oocytes with defective chromosome morphology. Interestingly, while all these features are shared between BPA and BPS, we also found distinct effects on the meiotic machinery where only BPA altered the kinetics of meiotic recombination suggesting that the two compounds also elicit distinct germline responses. RNA-seq and pathway analysis will reveal the molecular basis for the similarities and differences between BPA and BPS.

Poster Abstracts

P1

Abstract Withdrawn.

P2

***In Vitro* Assessment of Genotoxic Effects of *Ginkgo Biloba* Leaf Extract and Its Eight Constitutes.** Mei N[1], Lin H[1], Guo X[1], Manjanatha MG[1], Moore MM[1,2]. [1]National Center for Toxicological Research, Jefferson, AR, United States, [2]ENVIRON International Corporation, Little Rock, AR, United States.

Ginkgo biloba has been used for many thousand years as a traditional herbal remedy and its extract has been consumed for many decades as a dietary supplement. *Ginkgo biloba* leaf extract is a complex mixture with many constituents, including flavonol glycosides and terpene lactones. The National Toxicology Program 2-year cancer bioassay found that *Ginkgo biloba* leaf extract targets the liver, thyroid gland, and nose of rodents; however, the mechanism of *Ginkgo biloba* leaf extract-associated carcinogenicity remains unclear. In the current study, the *in vitro* genotoxicity of *Ginkgo biloba* leaf extract and its eight constituents was evaluated using the mouse lymphoma assay (MLA) and Comet assay. The underlying mechanisms of *Ginkgo biloba* leaf extract-associated genotoxicity were explored. *Ginkgo biloba* leaf extract, quercetin, and kaempferol resulted in a dose-dependent increase in the mutant frequency and DNA double-strand breaks. Western blot analysis confirmed that both quercetin and kaempferol activated the DNA damage signaling pathway with increased expression of γ-H2AX and phosphorylated Chk2 and Chk1. In addition, *Ginkgo biloba* leaf extract produced reactive oxygen species and decreased glutathione levels in L5178Y cells. Loss of heterozygosity analysis of mutants indicated that *Ginkgo biloba* leaf extract, quercetin, and kaempferol treatments resulted in extensive chromosomal damage. These results indicate that *Ginkgo biloba* leaf extract and its two constituents, quercetin and kaempferol, are mutagenic to the mouse L5178Y cells and induce double-strand breaks. Quercetin and kaempferol likely are major contributors to *Ginkgo biloba* leaf extract-induced genotoxicity.

P3

Evaluation of Bacterial Mutation Historical Positive Control Data. Dakoulas EW, Wagner III VO. BioReliance Corporation, Rockville, MD, United States.

OECD guideline 471 has required presentation of historical control data in reports since its adoption in 1997. This paper evaluates this laboratory's historical positive control data collected for studies conducted in 2010 through 2012. The tester strains included are TA98, TA100, TA1535, TA1537 and WP2 *uvr*A. Mean, standard deviation, minimum value, maximum value, coefficient of variation, and numbers of observations are presented for the positive controls for each of the above strains in both the presence and absence of S9 activation. These parameters were compiled monthly for individual counts, replicate counts and fold increases. In addition, a distribution of revertants per plate (individual and replicate) was complied for each strain, both with and without metabolic activation. Each strain exhibits a characteristic number of mean revertants that are variable from trial to trial but over a three year period and annually exhibit a consistent mean and SD. For each strain, the mean revertants per plate were also consistent month to month with no indication of a seasonal pattern. The mean revertants for each strain across individual counts and across replicate counts are comparable, with generally increasingly higher values in the 20- and 60-minute preincubation method. Each strain exhibits a characteristic variability that is smaller across replicate counts than across individual counts and this variability is consistent throughout the year and from year to year. Using a 95% confidence limit provides a more realistic assessment of the typical range of revertants than simply using minimum or maximum values.

P4

Combination of Two Tobacco Blend Technologies to Reduce Tobacco Smoke Particulate Matter Toxicant Yields: Assessment in the Ames and *In Vitro* Micronucleus Test. Crooks I, Dillon D, Dalrymple A, Scott K, Meredith C. British American Tobacco, Southampton, United Kingdom.

Tobacco smoke contains over 6000 constituents; approximately 150 of these are identified as toxicants. Technologies exist that modify the tobacco blend to reduce smoke toxicants, which include tobacco sheet substitute (TSS) to dilute toxicants in smoke, and blend treated tobacco (BT) to decrease levels of mutagenic precursors. BT and TSS were combined into an experimental cigarette (EC), which was compared to a control cigarette (CC), with a standard cellulose acetate filter and no blend technologies. The EC and CC were smoked to Health Canada puffing parameters (55 mL puff volume, over 2 s, every 30 s). The resulting particulate matter was subjected to an Ames test and an *in vitro* micronucleus test. Significant decreases in bacterial mutagenicity were observed in TA98 and TA100 relative to the CC ($p \leq 0.05$). In TA1537 overall, there were no differences between the EC and CC. Significant decreases in micronuclei induction ($p \leq 0.05$) were also observed with the EC. In conclusion, the data demonstrates that cigarettes that contain technologies to reduce toxicants can be developed without introducing additional genotoxic hazards as assessed by the Ames and *vitro* micronucleus tests. In some Ames strains and the micronucleus assay, consistent reductions in responses with the EC were observed.

P5

PPAR-α and PPARGC-1β Gene Expression in Gestational Diabetes Mellitus: No Relationship with Newborn Obesity Predisposition. Silveira MAD[1], Marcondes JPC[1], Prado RP[1], Andrade PFB[1], Luperini BCO[1], Rudge MVC[2], Salvadori DMF[1]. [1]OMICS, Laboratory of Toxicogenomics & Nutrigenomics, Department of Pathology, Botucatu Medical School, UNESP, São Paulo State University, Botucatu, SP, Brazil, [2]Department of Gynecology and Obstetrics, Botucatu Medical School, UNESP, São Paulo State University, Botucatu, SP, Brazil.

Obesity is a multifactorial disease involving complex interactions between genetic and environmental factors. However, the increased incidence, early onset, and severity of this disease, are still not well understood. Several findings have demonstrated that *in utero* stressors (cigarettes, diabetes, alcohol consumption, etc.) can promote transcriptional changes that modulate fetal development and predispose to later development of diseases, including obesity. The aim of this study was to evaluate whether PPAR-α and PPARGC-1β gene expression in placenta cells (fetal and maternal sides) and umbilical cord blood of newborns from mothers with Gestational Diabetes mellitus (GDM) can be used as early biomarkers for obesity development. The study included healthy pregnant (n = 10) and GDM (n = 10) women and their respective newborns (n = 10 from each pregnant group). Additionally, adult obese (n = 10) and eutrophic (n = 10) subjects were included as reference populations. Results showed no statistically significant differences in the PPAR-α and PPARGC-1β expression among the groups. However, the study population will be increased in order to improve the statistical analysis. Financial Support: FAPESP and CNPq.

P6

Comparison of JAK1 Inhibitors Using Whole Blood and Isolated Human Peripheral Blood Lymphocytes in the *In Vitro* Micronucleus Test. Hurtado SB[1], Moy ML[1], Wells MM[1], Harstad EB[2], Farabaugh CS[1]. [1]WIL Research, Skokie, IL, United States, [2]Genentech, Inc., South San Francisco, CA, United States.

The human peripheral blood lymphocyte (HPBL) micronucleus screen evaluates the ability of compounds to induce chromosome breakage (clastogenicity) and chromosome loss or gain (aneugenicity). Micronuclei are formed in the cytoplasm when chromosome fragments or whole chromosomes do not migrate with the rest of the chromosomes during cell division. This study compares the use of whole human venous blood (WB) to isolated human peripheral blood lymphocytes (IL) in the detection of micronuclei in the absence of a rat liver metabolic activation system (S9 fraction). Multiple concentrations of novel JAK1 inhibitors, the positive control Mitomycin C (MMC) and vehicle control dimethylsulfoxide (DMSO) were evaluated for cytotoxicity and the presence of micronuclei (MN) using both WB and IL. An increase in MN was considered positive if >6 MN were observed per culture and the response was 2 fold higher than the vehicle control. For both WB and IL, a positive response was observed with MMC at a concentration of 0.1 µg/mL, demonstrating the ability of the systems to detect MN. Cytotoxicity in the presence of the JAK1 inhibitors was comparable between both methods, with some variation when the cytotoxicity curve appeared steep. The JAK1 inhibitors were considered positive for inducing micronuclei in both the WB and IL methods in all compounds, indicating the two methods are comparable. The isolated lymphocyte format allows for smaller culture sizes and less test article making it an ideal method for screening for lead candidate selection, impurity or metabolite testing.

P7

Genotoxic Potential of Omeprazole on Human Peripheral Blood Lymphocytes. Kutsar V, LaFollette S, Gothke S, Wanner J, Vaglenov A. The University of Findlay, College of Pharmacy, Findlay, OH, United States.

Omeprazole (CAS # 73590-58-6) is one of the most commonly used proton pump inhibitor. This drug has indicated negative results by the Ames test (Mc Kena et al. 2008) and positive results by unscheduled DNA synthesis (UDS) in rat primary hepatocytes and gene mutations on mouse lymphoma thymidine kinase (TK) locus (IARC 1987; Martelli et al. 1998; The carcinogenic Potency Database http//potency, Berkely.edu; Toxnet), http://www.nlm.nih.gov. The micronucleus rate in lymphopcytes also increased in patients treated with omeprazole (Sinues et al, 2004). The long-term carcinogenic assays performed on rats exhibited strongly positive results (Mereto et al. 1993; Physicians' Desk Reference, 5th ed, 2005). The aims of this investigation are to evaluate the *in vitro* genotoxic effect of omeprazole through evaluation of DNA damage on human peripheral blood lymphocytes by using cytokinesis-block micronucleus (CBMN). The results suggest positive support for genotoxicity by the cytokinesis-block (CBMN) assay. Also, preliminary results through alkaline comet SCGE suggest that omeprazole acts as a genotoxicant.

P8

Investigation on Genotoxic Effects of Olanzapine. LaFollette S, Kutsar V, Gothke S, Vaglenov A. The University of Findlay, College of Pharmacy, Findlay, OH, United States.

Data on the genotoxic potential of the antipsychotic drug olanzapine's (CAS # 132539-06-1) is scarce. This drug has indicated negative results for the Ames test, *in vitro* unscheduled DNA synthesis (UDS) in rat primary hepatocytes, gene mutations on mouse lymphoma thymidine kinase (TK) locus, chromosomal aberrations in Chinese hamster ovary cells, as well as *in vivo* on SCE derived from human lymphocytes (Balbi A et al., 1980; Physicians' Desk Reference, 59 ed., 2005; Togar et al., 2011; Turkez et al., 2010). Other nonhuman models with different genetic endpoints have also confirm that olanzapine may not cause mutations (Brambilla et al., 2009, 2009). The long-term carcinogenic assays performed on rats and mice exhibited strongly positive results (Physicians' Desk Reference, 59 ed., 2005). The purpose of this investigation are to evaluate *in vitro* the possible genotoxic effect of olanzapine on human peripheral blood lymphocytes. The results indicated that olanzapine induce a positive response through the cytokinesis-block cytome CBMN assay and positive through the alkaline comet (SCGE) assay.

P9

Genotoxic Activity of Three Fungi Extracts. Kutsar V, LaFollette S, Vasquez M, Edelbrock M, Walker D, Vaglenov A. The University of Findlay, College of Pharmacy, Findlay, OH, United States.

The use of various fungi for primary health care has progressively increased worldwide in recent years. Many fungi and their respective secondary metabolites have anticarcinogenic effects, that make fungi extracts and products effective in the treatment of different type of cancers, as well as fungal and viral infections (McMorris et al., 2007; Schneider et al., 2008; Yeo et al., 2007; Zhang et al., 2007). The genotoxic activity of three *Epicocum nigrum* extracts (#590, #668, and #672), candidates for future isolation of active sub-ingredients, was tested through cytokinesis-block cytome assay (CBMN) and by alkaline Single ell Gel Electrophoresis (SCGE). The extracts 590, 668, and 672 have a weak genotoxic effect that were evaluated through CBMN assay. The genotoxic activity of the investigated extracts were also measured by alkaline Single Cell Gel Electrophoresis Assay (SCGE). All three extracts have genotoxic activities, but the extract #590 solely resulted in strong genotoxic effect similar to that of the positive control (H_2O_2).

P10

The Effects of 2-Acetylaminofluorene and Its Genotoxic Metabolites in a 3-D Reconstructed Human Skin Model As Measured in the Comet and Micronucleus Assays. Downs TR[1], Posgai R[2], Barnett B[2], Pfuhler S[1]. [1]Procter and Gamble Co, Mason, OH, United States, [2]Advanced Testing Labs, Cincinnati, OH, United States.

Development and validation of *in vitro* genotoxicity assays as "tier 2" assays to follow-up positive results from the current *in vitro* test battery has focused on using 3-D reconstructed human skin models in the Comet and micronucleus assays. These assays more realistically represent the exposure of dermally-applied chemicals such as cosmetic ingredients. Validation of these assays includes the ability to accurately detect chemicals that are not genotoxic themselves, but are converted into mutagenic and carcinogenic metabolites. 2-Acetylaminofluorene (2-AAF) is a carcinogen when administered dermally, orally, or by intraperitoneal injection in rodents and shows positive effects in some *in vitro* assays primarily after metabolic activation. N-hydroxy-2-acetylaminofluorine (N-OH-2-AAF) and N-hydroxy-2-aminofluorine (N-OH-2-AF) are genotoxic metabolites of 2-AAF which act via formation of DNA adducts. These compounds were topically applied to the EpiDerm™ skin model and DNA damage was measured in the Comet and micronucleus (MN) assays. 2-AAF itself did not increase DNA damage in the Comet assay or the percentage of keratinocytes with MN when administered in multiple (2-3) applications at 24h intervals. The same results were found with N-OH-2-AAF for MN while a single (3h) exposure to N-OH-2-AAF resulted in a significant dose-related increase in DNA damage in the Comet assay. A significant increase in the MN assay was only obtained with the highly reactive N-OH-2-AF following 72h of exposure. The results suggest that in a human skin model, the type of DNA damage caused by the 2-AAF metabolites may be more efficiently detected in the Comet assay than the MN assay.

P11

Qualification of the Comet Assay for Use in Human Biomonitoring. Pant K[1], Bruce SW[1], Springer S[1], Lawlor TE[1], Aardema MJ[1,2]. [1]BioReliance by SAFC, Rockville, MD, United States, [2]Marilyn Aardema Consulting, Fairfield, OH, United States.

The Comet assay can be used for biomonitoring of DNA damage in blood and bone marrow cells from patients participating in clinical trials. The method was initially qualified by storing healthy volunteer's blood in the refrigerator for up to 72 hours to mimic shipment from a clinical trial, then performing the comet assay. Results indicated that storage of blood at 2 to 8°C didn't increase % tail DNA compared to fresh blood samples. Bone marrow was qualified by storing rat bone marrow for up to 72 hours at 2 to 8°C. With the bone marrow samples, the % clouds in the samples increased in the refrigerated samples, however, the % tail DNA did not significantly increase. Having a robust historical control database is important in establishing validity and interpreting the results of the Comet assay. To this end, blood from healthy volunteers was treated with vehicle control saline or positive control methyl methanesulfonate for one hour to establish a control database for use in analysis of patient samples. Results from studies over five years (more than 100 samples from healthy volunteers) indicate the % tail DNA historical control for normal human blood is 1.4 ± 0.67 and for positive control treated samples is 18.34 ± 7.20. These data are routinely used to compare to patient samples taken at different time-points during clinical trials that are shipped to BioReliance at 2 to 8°C with temperature monitoring, where slides are prepared immediately.

P12

Evaluation of DNA Repair Center Dose- and Time-Dependence after Treatment with Chemicals Causing Linear and Nonlinear Dose-Response Curves for Micronuclei. Sun B[1], Ross S[1], Trask J[1], Carmichael PL[2], Adeleye Y[2], Andersen ME[1], Clewell R[1]. [1]The Hamner Institutes for Health Sciences, Durham, NC, United States, [2]Unilever, SEAC, Bedfordshire, United Kingdom.

The dose-dependence of micronuclei induction (MN) was evaluated in human p53-competent cells (HT1080) across a wide range of doses (18 doses, 3 orders of magnitude) for 8 chemicals that cause different types of DNA damage. Neocarzinostatin (NCS; γ-irradiation mimic), methyl methanesulfonate, ethyl nitrosourea (MMS, ENU; alkylating agents), H_2O_2, (oxidative damage), quercetin and curcumin (QUE, CUR; oxidative polyphenols), exhibited threshold-like dose-response curves, while etoposide (ETP; topo II inhibitor) and mitomycin C (MMC; DNA crosslinker) were more linear. DNA repair centers (DRCs) are aggregation sites of repair proteins that form at sites of DSBs and replication stress. We developed a high content assay using confocal microscopy to quantitate DRCs containing two essential proteins - p53BP1 and p-H2AX. Dose-response and time course measurements were performed with two chemicals that showed linear (ETP) or nonlinear (NCS) MN induction. ETP induced DRCs that were not resolved within 27 hr even at low concentrations. NCS showed a very different DRC response. At low doses (5 ng/mL NCS), DRCs were formed quickly and were resolved within 3 hr. At higher doses, however, the number of DRCs remained high at 27 hr. These results indicate that cells can more efficiently repair DSBs induced by NCS than ETP at low doses, and this greater repair efficiency may explain the different shapes of the dose response curves for the two chemicals. We are currently evaluating the time and dose-dependence of DRCs with additional chemicals to better define the relationship between DRC kinetics and MN dose-response trends.

P13

Cells Deficient in the FANC Repair Pathway Are Hypersensitive to Isopropyl Methanesulfonate. Hashimoto K[1,2], Swenberg J[1], Nakamura J[1]. [1]Department of Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, NC, United States, [2]Drug Safety Research Laboratories, Takeda Pharmaceutical Co. Ltd, Fujisawa, Kanagawa, Japan.

Sulfonic acid can react with methanol, ethanol, or isopropanol to form genotoxic impurities (GIs) known as sulfonate esters. Isopropyl methane sulfonate (IPMS) is a potential contaminant in pharmaceutical-grade medications. However, little is known about IPMS compared to other sulfonate esters, such as methane sulfonate (MMS) and ethyl methane sulfonate (EMS) in terms of genotoxicity. The present study serves to compare the genotoxic profile of IPMS with that of MMS and EMS. The study was conducted using chicken DT40 cell lines with targeted mutations in various DNA repair genes. IPMS was more toxic to FancD2-deficient cells compared to parental DT40 cells, while MMS and EMS were comparably toxic to both WT and FancD2-deficient DT40 cells. These results indicate that the FANC pathway is critical in tolerating DNA damage caused by IPMS. All of these GIs alkylate DNA at the O^6 position of guanine, leading to the formation of O^6-alkylguanine. Because the O^6-alkylguanine adducts are suspected to be repaired by O^6-methylguanine-DNA methyltransferase (MGMT), we addressed the effects of an MGMT inhibitor, O^6-benzylguanine (O^6BG), on the DNA damage response by IPMS, MMS, or EMS. While O^6BG potentiated the DNA damage response to MMS and EMS in FancD2-deficient cells, IPMS caused a similar extent of DNA damage response regardless of O^6BG. These results strongly suggest that MGMT can repair both O^6-methylguanine and O^6-ethylguanine adducts but not O^6-isopropylguanine adducts. Although these GIs are unlikely to cause DNA-DNA or DNA-protein crosslinks, all O^6-alkylguanines investigated in this study were subject to a FancD2-mediated DNA repair pathway.

P14

Use of *N*-Methyl-2-pyrrolidone (NMP) As a Solvent in the *In Vitro* Cytogenetic Assay. Roy S, Wang K, Khasamba G. BioReliance by SAFC, Rockville, MD, United States.

It is necessary to evaluate the solubility of the test chemical not only in various solvents but also after adding the required dilutions to culture medium. The most common solvents used in the *in vitro* cytogenetic studies are water, saline, DMSO, acetone, and ethanol. With the introduction of combinatorial chemistry the properties of new chemical entities has shifted towards higher molecular weight and increasing lipophilicity that results in decreasing in solubility property. It has been observed that some of the high molecular weight compounds, particularly polymers, are insoluble in common solvents used in the *in vitro* cytogenetic assay. Therefore, it is important to explore alternate solvents by evaluating the compatibility with the test system and their impact on the final endpoints of the test. We evaluated suitability of *N*-Methyl-2-pyrrolidone (NMP) as a solvent in the *in vitro* cytogenetic assay using CHO cells and HBPL, commonly used *in vitro* test systems in the genotoxicity study for regulatory submission. NMP is dipolar solvent and miscible in the cell culture medium. Neat NPM was tested in two test systems at 0.25%, 0.5%, 1%, and 2% (v/v). Cultures were processed for parameters like cytotoxicity (cell growth inhibition and mitotic index) and genotoxicity (chromosome aberrations). NPM was found cytotoxic and genotoxic at ≥1% dose level in both test systems. However, at 0.25% and 0.5%, the cytotoxicity and genotoxicity was comparable to the untreated control. Our result indicates, when needed, the NMP may be used as a solvent *in vitro* cytogenetic assay at ≤0.5% (v/v).

P15

Prediction of Genotoxicity of Nano Metal Oxides by Computational Methods: A New Decision Tree QSAR Model. Golbamaki Bakhtyari N[1], Golbamaki Bakhtyari A[1], Benfenati E[1], Cronin M[3], Rasulev B[2], Leszczynski J[2]. [1]Mario Negri Institute for Pharmaceutical Research, Milan, Italy, [2]Jackson State University, Jackson, MS, United States, [3]Liverpool John Moores University, Liverpool, United Kingdom.

Due to the rapid growth in the nanotechnology industry, there is an urgent need to define a hazard identification and risk management strategy for nanomaterials. An increasing number of nanomaterial safety studies include an assessment of genotoxicity as part of the overall risk evaluation. Presently, the genotoxicity assays that are employed for nano metal oxides assessments are adaptations of chemical genotoxic assays such as Ames test, *in vitro* micronucleus assay, and single cell electrophoresis or comet assays. These assays are developed to evaluate bulk materials and they are not equipped for assessing the nanomaterials. Developing rapid methods for predicting the toxic behaviour and environmental impact of these nanoparticles is therefore important and timely. Computational predictive toxicology to screen and prioritize chemicals in both the development and safety assessment stages are promoted by different chemical legislations. This work summarizes recent genotoxicity studies on metal oxide nanoparticles collected from the literature. A data base of 15 metal oxide nanoparticles have been created based on the experimental data gathered. A well constructed cluster model of the nanoparticle for the calculation of the properties has been employed. The structures were then used as input for quantum-mechanical calculations at the semi-empirical PM6 level. A predictive model based on Classification and Regression Tree (CART) was developed. The model showed a good correlation between selected physico-chemical descriptors and genotoxicity, which can be useful in predicting the genotoxicity of new and untested metal oxide nanoparticles.

P16

Assessment of Carboxyl Graphene Nanoplatelet Toxicity in Human B-Lymphoblasts. Petibone DM[1], Ding W[1], Mustafa T[2,3], Lafont A[2], Xu Y[2], Watanabe F[2], Casciano D[2], Dobrovolsky V[1], Biris AS[2], Morris SM. [1]Division of Genetic and Molecular Toxicology, National Center for Toxicological Research, Jefferson, AR, United States, [2]Center for Integrative Nanotechnology Sciences, University of Arkansas at Little Rock, Little Rock, AR, United States, [3]Biology Department, College of Science for Women, University of Baghdad, Bagdad, Iraq.

Carboxyl nano-graphene (G-COOH) has promising technological and biomedical applications with the potential to improve quality of life. However, these G-COOH implementations present avenues for human exposures, and as with any new technology, the risks to public health are unknown. To define these risks, we evaluated G-COOH cytotoxicity and genotoxicity in human B-lymphoblasts. Relative increases in cell count and plating efficiencies measured cell growth and survival, respectively. Evaluation of cell membrane integrity and apoptosis used propidium iodide and annexin-V. The standard, EndoIII and hOGG1 modified comet assays determined G-COOH induced DNA strand breaks and oxidative DNA damage. In addition, the micronucleus (MN) assay detected clastogenicity and whole chromosome fluorescence *in situ* hybridization (FISH) of metaphase cells determined structural chromosome damage. Dihydroethidium (DHE) served as a molecular probe to detect reactive oxygen species (ROS). G-COOH exposures resulted in decreased B-lymphoblast growth and survival. Upon the initial exposure, G-COOH induced cell membrane damage, followed by increased apoptotic levels. The standard comet assay revealed significant increases in DNA strand breaks following G-COOH exposure. The EndoIII and hOGG1 modified comet assays indicated significant increases in oxidative DNA damage at a 10 µg/ml G-COOH dose. FISH analysis revealed an increase in chromosome damage following G-COOH exposure. However, the MN was negative for G-COOH induced clastogenicity at all G-COOH doses tested. At the same time, DHE analysis indicated that G-COOH induced dose-dependent increases in ROS levels. Results from this study suggest that cell membrane damage, induction of ROS and oxidative stress are possible mechanisms of G-COOH toxicity.

P17

Genome Profiling of Yeast Resistance to Aflatoxin B_1 Reveals Significance of DNA Damage Tolerance Genes in Conferring Toxin Resistance. Fasullo MT[1], Cera CA[1], Bard J[2], Freedland J[1], Egner P[3], Begley T[1]. [1]State University of New York, Albany, NY, United States, [2]State University of New York at Buffalo, Buffalo, NY, United States, [3]Johns Hopkins University, Baltimore, MD, United States.

The mycotoxin aflatoxin B_1 (AFB_1) is the most potent liver carcinogen. A signature p53 mutation is found in AFB_1-associated hepatic tumors, suggesting that AFB_1 is a potent genotoxin. P450 enzymes convert AFB_1 into a highly reactive epoxide that forms N7-guanine DNA adducts. In CYP1A2-expressing budding yeast, AFB_1 is a weak mutagen but a potent recombinagen, and triggers the formation of Rad51 foci. Microarray analysis reveals that both DNA repair and stress response genes are upregulated. To elucidate the functional significance of transcriptional induction, we profiled the yeast genome for AFB_1 resistance, using state-of-the-art next generation sequencing to identify molecular barcodes. We introduced the human CYP1A2 into ~90% of the deletion library, and pooled samples were exposed to 50 µM and 100 µM AFB_1 for 20 hrs. We identified genes that confer resistance to AFB_1 by barcode sequencing, and grouped resistance genes into gene ontology groups using FunSpec and SGD analysis. Approximately 4300 ORFs were identified in the screen. Individual resistance genes were confirmed by survival curves and FACS, using a wild-type diploid containing the gene encoding green fluorescent protein (GFP) inserted at the HO locus. DNA metabolism genes include those functioning in DNA damage tolerance, checkpoint recovery and replication fork maintenance, emphasizing the potency of the mycotoxin to trigger replication stress. Additional genes include those that function in autophagy, TOR signaling, cellular growth and cytoskeletal architecture. The ultimate aim will be to identify corresponding mammalian genes. The yeast libraries will be valuable for additional high-throughput studies using other metabolically-activated carcinogens.

P18

Analysis of Polymorphisms of *GSTM1* and *GSTT1* Genes and Transcriptome in Public Health Agents Occupationally Exposed to Pesticides. Franco FC[1], Godoy FR[1], Alencar M[2], Marques AA[1], Batista MP[1], Melo CO[1], daSilva CC[2], Cruz AD[2], Silva DM[1]. [1]Universidade Federal de Goiás, Goiânia, Goiás, Brazil, [2]Pontifícia Universidade Católica de Goiás, Goiânia, Goiás, Brazil.

Pesticides belong to a chemical category developed to eliminate pests, although the advantages, some of these are associated with risk of the health. Occupational exposure to pesticides is particularly worrying to public health agents in eradication campaigns, whereas, the combat vector is still the solution to prevent dengue epidemics. The aim of this study (case-control) was the evaluation of occupational exposure to pesticides used to eradicate dengue vectors by public health agents in Aparecida de Goiania. We applied a questionnaire for lifestyle and professional knowledge after signing a consent form. We used commercial kits to extract nucleic acids; the polymorphisms of *GSTM1* and *GSTT1* were analyzed by qPCR and the transcriptome by microarray. Both groups are composed of 125 people, approximately half male and half female, in which 14% of exposed and 10% of controls are smokers. Regarding the use of PPE 83% confirm the use of gloves and mask at least sometimes and 90% use boot. Analysis of polymorphisms on *GSTM1* and *GSTT1* showed no significance difference (p=0.498) by chi-squared test between case and control groups. Indicating no affect in the integrity of these detoxification genes. For the microarray analysis were selected 8 members divided into low (<12 months) and longer exposure (>108 months), 53,617 genes expression analyzed showed no significant difference between the groups, according to the FDR test. There is no real quantification of the contact with the pesticide despite of the exposure time, so the next steps will be to compare exposed groups to controls.

P19

Developing an Integrated "*In Vitro*" Carcinogenicity Tool. Wilde E, Seager A, Johnson G, Doak S, Jenkins G. Swansea University, Swansea, United Kingdom.

A major concern with *in vitro* genotoxicity assays is increased misleading-positive results. This project, funded by the National Centre for the Replacement, Refinement and Reduction of Animals in Research, aims to improve *in vitro* safety assessment. The procedure involves testing effects of known carcinogens on cell genotoxicity, phenotype and signalling. TK6 cells were treated for 1.5 cell-cycles with Methyl-Methanesulfonate (MMS), N-Nitroso-N-Methylurea (MNU), Methyl-Carbamate (non-genotoxic) and 2,4-Dichlorophenol. Genotoxicity was then analysed using the cytokinesis-block micronucleus assay. Changes in cell-cycle and cell morphology were detected by image analysis (InCell-Analyzer 2000). Western blotting was then carried out to assess expression of proteins p53 and phospho-p53, and gene expression analysed using a DNA Microarray with chemicals MMS and Methyl-Carbamate. Significant genotoxicity was initiated by MMS, MNU and 2,4-Dichlorophenol at 0.7, 0.3 and 30 µg/mL in the micronucleus assay (Dunnett's t-test, $p < 0.05$). A clear dose-dependent increase in proteins p53 and phospho-p53 was seen in response to MMS and MNU, whilst no change for Methyl-Carbamate. Cell imaging showed a dose-dependent G2/M cell-cycle block for chemicals MMS and MNU, increasing average nuclear area and nuclear perimeter. The increase in genotoxicity shown by MMS and MNU abides with the increase in p53 and phospho-p53, and also the cell cycle block, since these proteins are known to cause G2 cell-cycle arrest in order to initiate DNA repair or apoptosis. Linking these sets of data will indicate how the effects of specific compounds relate to their mechanisms of action, improving the identification of potential carcinogens *in vitro.*

P20

Use of Human TK6 Lymphoblastoid and HepaRG Cell Lines for Genotoxicity Testing in a Flow Cytometry-Based *In Vitro* Micronucleus Assay. Swartz CD[1], Recio L[1], Green A[1], Buick J[2], Yauk CL[2]. [1]Integrated Laboratory Systems, Research Triangle Park, NC, United States, [2]Health Canada, Ottawa, ON, Canada.

Integrated Laboratory Systems (ILS) uses an automated flow cytometry-based MN assay in human TK6 cells as part of its portfolio of capabilities for Good Laboratory Practice-compliant regulatory safety assessment. The assay is performed in accordance with OECD Test Guideline #487 and ILS has an extensive historical database in TK6 cells. We have recently completed studies to optimize the metabolic activation system routinely used in the assay, testing both S9 concentration and a variety of induction agents. Arochlor-, phenobarbital/benzoflavone-, and ethanol -induced rat liver S9, and mixes of these, were tested at final concentrations of 2-10%. We determined that arochlor-induced S9 used at 2% in the S9 mix best captured the activity of the known genotoxins without causing excess cytotoxicity to the TK6 cells. In addition, we have developed and optimized the *in vitro* MN assay for use in HepaRG cells, a commercially available, metabolically competent human hepatocellular carcinoma cell line. The unique features of the HepaRG cell line make them suitable for high throughput screening and for metabolism and toxicity studies. In testing to date, the assay in HepaRG cells has shown 100% specificity, and approximately 50% sensitivity in detecting known genotoxic compounds. Cytotoxicity of the various compounds in HepaRG cells has been variable compared to that seen in TK6 cells. Results of optimization experiments and genotoxicity testing will be presented.

P21

"Automated *In Vitro* Micronucleus Scoring," Flow Cytometry V Image Analysis. Verma J[1], Rees B[1], Jenkins G[1], Bryce S[1,2], Johnson G[1]. [1]Swansea University, Swansea, Wales, United Kingdom, [2]Litron Laboratories, Rochester, NY, United States.

The *in vitro* micronucleus assay (MN assay) is a globally approved test for detecting genotoxic agents, within the environment and in pharmaceuticals. In this assay, the quantitative index on DNA damage is obtained by scoring micronuclei (MN) in cultured mammalian cells. Scoring such MN manually can be subjective, tiresome, and time consuming. Therefore, it is essential to replace the conventional manual MN scoring platform with semi or fully automated scoring platforms. This project aims to evaluate the sensitivity and suitability of the fully automated flow cytometry based MN assay (MicroFlow kit, Litron Laboratories USA) for dose response analysis, and compare it to an automated image analysis (Metafer) method. Human lymphoblastoid TK6 cells were treated with methyl methane sulfonate (Clastogen), Carbendazim (aneugen) and Ochratoxin A (weak genotoxic carcinogen). Different treatment strategies included a 4 hour or 30 hour treatment time, with or without recovery. A clear dose dependent induction in MN was detected by both MicroFlow and Metafer for MMS, Carbendazim and Ochratoxin A. Furthermore, cell cycle analysis with MicroFlow revealed a dose dependent G2/M cell cycle arrest for MMS and Ochratoxin A, whereas Carbendazim caused a significant G1 block. The result show that the MicroFlow approach an improved, sensitive and a comprehensive MN analysis approach compared to automated image analysis (Metafer). Both MicroFlow and Metafer techniques are rapid, high content and automated scoring platforms suitable for scoring MN in the *in vitro* MN assay.

P22

Mode-of-Action Investigations in Support of Secondary Mechanisms Using TK6 Cells and Flow Micronucleus. Sullivan A[1], Posgai R[2], Pfuhler S[1]. [1]The Procter & Gamble Company, Mason, OH, United States, [2]Advanced Testing Laboratory, Cincinnati, OH, United States.

Traditional genotoxicity risk assessment is limited to a yes/no readout, however, there has been a recent transition away from this system towards a "point of departure" (PoD)-based model. The PoD model may help to distinguish primary genotoxic effects from secondary mechanisms such as oxidative stress and inflammation that induce indirect DNA damage. The initial studies shown here use the TK6 cell line and flow cytometric measurement of micronuclei to increase knowledge about the pathways involved in direct and indirect modes of action (MoA). The compounds of choice were the flavonoid quercetin, which despite being nongenotoxic *in vivo* in OECD guideline studies, shows mutagenicity and genotoxicity *in vitro* and mitomycin C as a DNA-reactive control. Specifically, cells were treated for four hours with seven doses of quercetin (0.9-10 ug/ml) and were either pre-treated with L-buthionine-sulfoximine (BSO) which blocks glutathione biosynthesis, a key part of a cell's natural antioxidant defense system or co-treated with ascorbic acid, a ROS scavenger. Testing quercetin alone demonstrates a non-linear response showing a sharp increase at highly cytotoxic doses. The BSO pre-treatment of the cells shifts the curve to the left, demonstrating a more linear response. Additionally, concurrent treatment with ascorbic acid was able to abolish genotoxicity to a large extent. The results shown here support oxidative stress as an important factor in quercetin genotoxicity. Ultimately, it is our goal to continue assessing various MoA's expected to result from key representative chemical classes.

P23

Evaluation of Repeated Dose Liver and Gastrointestinal Tract Micronucleus Assay Using Young Adult Rats (IV): Summary of Collaborative Study by CSGMT/JEMS.MMS. Hamada S[1,20], Ohyama W[2], Takashima R[1], Shimada K[3], Matsumoto K[4], Kawakami S[5], Uno F[6], Sui H[7], Shimada Y[8], Imamura T[9], Matsumura S[10], Sanada H[11], Inoue K[12], Muto S[13], Ogawa I[14], Hayashi A[15], Takayanagi T[16], Ogiwara Y[17], Maeda A[18], Okada E[2], Terashima Y[19], Takasawa H[1], Narumi K[2], Wako Y[1], Kawasako K[1], Morita T[20], Kojima H[20], Honma M[20], Hayashi M[6]. [1]LSI Medience Corporation, Ibaraki, Japan, [2]Yakult Honsha Co., Ltd., Tokyo, Japan, [3]Astellas Pharma Inc., Osaka, Japan, [4]Astellas Research Technologies Co., Ltd., Osaka, Japan, [5]Asahi Kasei Pharma Corporation, Shizuoka, Japan, [6]Public Interest Incorporated Foundation, Biosafety Research Center, Shizuoka, Japan, [7]Food and Drug Safety Center, Kanagawa, Japan, [8]Hokko Chemical Industry Co., Ltd., Kanagawa, Japan, [9]Ina Research Inc., Nagano, Japan, [10]Kao Corporation, Tochigi, Japan, [11]Kaken Pharmaceutical Co., Ltd., Shizuoka, Japan, [12]Maruho Co., Ltd., Kyoto, Japan, [13]Mitsubishi Tanabe Pharma Corporation, Chiba, Japan, [14]Nissan Chemical Industries, Ltd., Saitama, Japan, [15]Shin Nippon Biomedical Laboratories, Ltd., Kagoshima, Japan, [16]Suntory Business Expert Limited, Osaka, Japan, [17]Taisho Pharmaceutical, Co., Ltd., Saitama, Japan, [18]Toray Industries Inc., Kanagawa, Japan, [19]Kissei Pharmaceutical Co., Ltd., Nagano, Japan, [20]National Institute of Health Sciences, Tokyo, Japan.

The repeated dose liver micronucleus (RDLMN) assay has a potential to detect genotoxic hepatocarcinogens that can be integrated into a general toxicological study. We have conducted a joint research in the Collaborative Study Group for the Micronucleus Test to assess the performance of the assay. 22 model chemicals including hepatocarcinogens were tested in RDLMN assays. As a result, 14 out of 16 hepatocarcinogens were positive in the RDLMN assay, including 9 genotoxic hepatocarcinogens, which were reported negative in the bone marrow MN assay. These outcomes show high sensitivity of the RDLMN assay to hepatocarcinogens. For the specificity, 4 of 6 non-liver targeted genotoxic carcinogens gave negative response in the RDLMN assay. In addition, we conducted gastrointestinal tract MN assays using 6 carcinogens. The MN assay using the glandular stomach, which is the first contact site to the test chemical, could detect the clastogenicity of 3 test chemicals including a stomach-targeted carcinogen. Recent activity of the collaborative study by CSGMT/JEMS.MMS will be also presented.

P24

Detection of Germ Cell Mutagens in the Muta™Mouse Transgenic Mutation Assay. Beevers C, Pearce G. Covance Laboratories Ltd, Harrogate, United Kingdom.

OECD test guideline 488 describes optimum study designs for determination of mutagenicity in a variety of rodent tissues. For male germ cells sampling times should be carefully selected to ensure the range of exposed cell types throughout germ cell development is sampled and that each stage targeted has had sufficient exposure. The guideline recommends one of two approaches for germ cell mutation assessment: 1) to dose for 28 days plus 3 days expression period and sample both developing germ cells from the seminiferous tubules and mature sperm from the cauda epididymis/vas deferens or 2) to dose for 28 days and sample mature sperm only a minimum of 7 weeks (mice) or 10 weeks (rat) after last treatment. Option 1 has significant advantages in terms of logistics, duration, cost of the study and integration with analysis of somatic cells. However, it has been questioned whether all stages of germ cell development are adequately covered by this design. We treated male Muta™Mice with water or ethylnitrosurea (ENU). Animals were treated orally for 28 consecutive days at 10 mg/kg/day. On Day 31 and Day 77, animals were necropsied and developing germ cells and mature sperm were isolated. Both cell types were examined for mutation in the *lacZ* transgene. An increase in mutant frequency (MF) was detected in developing germ cells but not mature sperm on Day 31. An increase in MF in mature sperm was only seen on Day 77, supporting the idea that the 28+3 day study design may not be optimal for robust detection of germ cell mutagens.

P25

How to Assess the Toxic, Reprotoxic, and Transgenerational Effect of Pollutants in *Drosophila melanogaster*? Arroyo E, Ramos P. Universidad Nacional Autónoma de México, DF, Mexico.

The use of various indicators in assessment of genotoxins is necessary for reducing the false negative responses and for understanding the effects in the medium and long term in populations exposed to uncontrolled conditions, such as environmental accidents. The death of organisms is an unambiguous response from exposure to contaminants, but what other responses can be quantified to establish the extent of their impact? How long should follow the assessment of their effects? Five biomarkers were used to evaluate: toxicity, reprotoxic damage and the transgenerational effect of Sodium Azide (SA) in *Drosophila*: Survival Index (SI), Sex Ratio (SR), Fertility (F), Progeny-per-male (PM) and Fecundity (Fe). Third instar larvae of *Drosophila* were fed with SA or distilled water. Recovered flies were counted and sexed to obtain the SI and SR. For each concentration, 15 males were mated with untreated females to produce the F1 which was counted and classified by sex to estimate the F and PM. Five couples from 8 families F1 were mated to produce the F2 and determine whether the parental exposure have effects on the F and Fe of unexposed progeny. In treated flies, high concentrations of SA were toxic, although the SR was unaffected. The exposure of parental males to SA modified their F and PM, but also has effect on Fe of their offspring. These results reinforce the need to modify the strategies to assess the *in vivo* impact of genotoxins in surviving organisms and their progeny. Acknowledgements: A Muñoz, H Rivas, B Hernández, DSCM.

P26

Aneuploidogenic Effects of Epirubicin in Somatic and Germinal Cells of Male Mice. Attia SM, Bakheet SA, Ahmad FS. Department of Pharmacology, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

The ability of the antineoplastic agents, epirubicin, to induce aneuploidy and meiotic delay in somatic and germinal cells of male mice was investigated by fluorescence *in situ* hybridization assay using labeled DNA probes and BrdU-incorporation assay, respectively. Mitomycin C and colchicine were used as a positive control clastogen and aneugen, respectively, and these compounds produced the expected responses. Using the fluorescence *in situ* hybridization assay with centromeric DNA probe for erythrocyte micronuclei showed that epirubicin is not only clastogenic but also aneugenic in somatic cells *in vivo*. By using the BrdU-incorporation assay it could be shown that the meiotic delay caused by epirubicin in germ cells was about 24 h. Disomic and diploid sperm were shown in epididymal sperm hybridized with DNA probes specific for chromosomes 8, X and Y after epirubicin treatment. The observation that XX- and YY-sperm significantly prevailed XY-sperm indicates missegregation during the second meiotic division. The results suggest also that earlier prophase stages contribute relatively less to epirubicin-induced aneuploidy. Both the clastogenic and the aneugenic potential of epirubicin can give rise to the development of secondary tumors and abnormal reproductive outcomes in cured cancer patients and medical personnel exposing to drug regimens that include epirubicin. Thus, genetic counseling of these patients should take place before the start of chemotherapy and should take the present results into consideration.

P27

Evaluation of Potential Sex Effects in the *Pig-a* Mutation Assay. Labash CB[1], Avlasevich SL[1], Carlson KM[1], Torous DK[1], Bemis JC[1], MacGregor JT[2], Dertinger SD[1]. [1]Litron Laboratories, Rochester, NY, United States, [2]Toxicological Consulting Services, Bonita Springs, FL, United States.

The induction of gene mutation at the X-linked reporter *Pig-a* was evaluated in female and male Sprague Dawley rats to determine whether sex-related differences would be evident in the frequency of reticulocytes (RET), mutant phenotype reticulocytes (RET^{CD59-}), or mutant phenotype erythrocytes (RBC^{CD59-}). Blood was collected and analyzed on days -4, 15, 29 and 46 after exposure to 0, 1, 5, or 25 mg/kg/day *N*-ethyl-*N*-nitrosourea (ENU) using a high throughput immunomagnetic separation- and flow cytometry-based method (MutaFlow®) to determine the mutant frequency. Additionally, samples collected on day 4 were analyzed for micronucleated reticulocyte (MN-RET) frequency (MicroFlow®). %RET were markedly higher in males compared to females on days -4 through 15. On day 4, 25 mg ENU/kg/day reduced mean %RET in both sexes, with no evidence of a Sex x Treatment interaction (two-way ANOVA). At the highest two dose levels, ENU exposure significantly increased RET^{CD59-} and RBC^{CD59-} frequencies in both sexes. At the high dose level, the absolute RET^{CD59-} and RBC^{CD59-} frequencies were somewhat lower for females compared to males. There was a significant Sex x Treatment effect on RET^{CD59-} but not RBC^{CD59-} frequency. The highest two doses caused similarly elevated %MN-RET frequencies. Although there were quantitative differences in RET and RET^{CD59-} frequencies, qualitative responses of *Pig-a* gene mutation were similar in males and females. The data support the use of both sexes in *Pig-a* gene mutation studies. Work in progress is evaluating whether the quantitative differences are related to the higher cell proliferation rate in young male rats.

P28

Pig-a Mutation Assay and Micronucleus Induction in Reticulocytes of Rats Exposed to Acrylamide, Aristolochic Acid, Chlorambucil, Melphalan, 1,3-Propane Sultone, and ThioTEPA. Elhajouji A, Al-Ghabban M, Hove T, O'Connell O, Martus HJ. Novartis Institutes for Biomedical Research, Basel, Switzerland.

Over the past several years there has been considerable amount of interest in the *in vivo* point mutation Pig-a assay. An inter-laboratory effort was launched to investigate the merits and limitations of this Pig-a gene based assay. Flow cytometric analysis of cells was based on the binding of anti-CD59 antibodies. A nucleic acid dye SYTO13 was used to differentiate between the two populations of peripheral erythrocytes. The frequency of CD59-negative reticulocytes (RETCD59-) and erythrocytes (RBCCD59-) served as phenotypic reporters of mutation at the Pig-a gene. Wistar Han rats were treated by oral gavage with six known mutagens: Acrylamide, Aristolochic Acid, Chlorambucil, Melphalan, 1,3-Propane Sultone and ThioTEPA following 3-day and 28-day dosing schedules. In addition to the Pig-a assay the incidence of micronucleated reticulocytes (MN-RET) was used to evaluate chromosomal damage. All chemicals with the exception of acrylamide showed a statistically significant dose dependant increase in MN-RET, RETCD59- and RBCCD59- frequencies in their respective studies in both 3 day and 28 day studies. The RETCD59- and RBCCD59- frequencies of the protracted studies achieved much higher values than the acute treatment study even though they elevated at a lesser rate and consisted of lower doses. This shows that the mutation occurs in an additive fashion and suggests that the Pig-a mutation happens in the haematopoietic stem cell. This study contributes towards the validation effort of the assay showing its reproducibility, portability, and sensitivity facilitating its integration into current toxicological studies.

P29

Data for the *Pig-a* Assay: *Pig-a* Assay in Female, Sensitivity to Detect the Initial Increase in Mutants, and *Pig-a* Assay with Preserved Blood Sample. Chikura S, Kimoto T, Okada KS, Kobayashi XM, Itano Y, Miura D, Kasahara Y. Teijin Pharma Limited, Tokyo, Japan.

The *Pig-a* assay is currently being investigated as a useful tool for evaluating *in vivo* mutagenicity. In Japan, the JEMS/MMS *Pig-a* collaborative study (HIS49/CD59 method, the RBC *Pig-a* assay and the PIGRET assay) is in progress. In this study, we investigated *Pig-a* assay in the following view points; i) availability in female rats, ii) sensitivity to detect the initial increase in mutants, and iii) feasibility with blood samples preserved under refrigerated condition (2-8°C). Male and female rats were administrated a single oral dose of ENU (10 and 40 mg/kg). Blood was sampled at 0, 3 and 5 days, 1, 2 and 4 weeks after the dosing and at autopsy. The RBC *Pig-a* and the PIGRET assays were performed with fresh and preserved samples. In RBC *Pig-a* and PIGRET assays, the *Pig-a* mutation frequency in female rats showed statistically significant increases from one week after the ENU dosing compared to pre-value, which were similar to results obtained in male rats. These results demonstrated *Pig-a* assay was available in both sexes. Compared to RBC *Pig-a* assay, an obvious increase in mutant cells were observed at 5 days after the ENU dosing by PIGRET assay. These results indicated the PIGRET assay could detect the increase in mutant cells at an early stage more than the RBC *Pig-a* assay. By comparing the data with fresh and preserved samples, we found RBC *Pig-a* and PIGRET assays were feasible with blood samples preserved under refrigerated condition for up to 7 days.

P30

Investigating the Pig-A Gene Mutation Assay As a Potential Biomarker in Patients with Esophageal Cancer: A Pilot Study. Haboubi H, Rees B, Thornton C, Johnson G, Jenkins G. Swansea University, Swansea, Wales, United Kingdom.

Introduction: Barrett's Esophagus (BE) is a premalignant condition affecting the lower esophageal mucosa, caused by chronic Gastro-Esophageal Refliux Disease (GERD). Noxious refluxate constituents in patients with GERD can induce reactive oxygen species and subsequent DNA damage, driving the progression of BE to Esophageal Adenocarcinoma (EA). The Pig-A gene mutation assay is a reproducible method for indirectly assessing genomic instability using flow-cytometric methodology. We postulate that blood cells circulating through the inflamed esophageal mucosa, exposed to mutagenic chemicals such as bile can be investigated using this assay. Methods: Blood-based cell lines were exposed to physiological carcinogens such as bile and the Pig-A mutant frequency measured. Subsequent *ex vivo* analysis of blood was undertaken in patients attending endoscopy with symptoms of GERD. Pig-A analysis of erythrocytes and leucocytes was performed and results correlated with histopathological analysis of esophageal biopsies as well as a detailed lifestyle questionnaire. Results: *In vitro* investigations confirmed the carcinogenicity of bile to blood-based cell lines, with increased mutant frequencies detected through the Pig-A gene mutation assay ($p<0.05$). Subsequent *ex vivo* erythrocyte analysis demonstrated no effect from patient age or gender on mutant frequency. Higher mutant frequencies were observed in EA patients compared to both normal GERD patients and those with Barrett's ($p<0.01$), but there was no significant difference between BE and normal controls. Conclusions: The application of this simple, noninvasive blood-based mutation frequency assay to patients with GERD suggests there may be a role for this assay as a biomarker for cancer.

P31

50 Shades of Pig-A: Investigating the Issues in Validating the *Ex Vivo* Pig-A Assay. Haboubi H, Rees B, Thornton C, Johnson G, Jenkins G. Swansea University, Swansea, Wales, United Kingdom.

Background: The Pig-A gene mutation assay was first described in the assessment of patients with paroxysmal nocturnal hemoglobinuria. This condition is caused by mutations to the Pig-A gene coding for GPI-anchors, which tether cell surface antigens such as CD55 and CD59. Mutational events affecting the Pig-A gene thus cause a GPI-anchor deficient phenotype, which can be detected indirectly using flow cytometry. The use of this assay in carcinogenicity testing, both *in vitro* and *in vivo* has gained popularity due to the low background mutant frequencies coupled to the fast, reproducible and high-throughput power of flow-cytometric methodology. However, issues remain in variation of methodology between centers, with the potential to influence mutant frequency levels. Methods: We investigated possible factors in validating the *ex vivo* Pig-A gene mutation assay. Blood-cell type, enrichment methodologies and gating strategies were all analyzed on 30 "healthy" controls to assess reported variability in Pig-A mutation frequency due to methodology differences rather than true mutational events. Results: Cell selection may be important in the use of the Pig-A mutation assay. Different enrichment techniques for leucoyte populations can falsely elevate reported mutation frequencies. Furthermore, flow-cytometry settings, namely flow rate, voltage and gating selection all have the potential to influence mutation frequencies. Conclusions: Standardizing methodology for the analysis of Pig-A mutants in blood cell lines will assist in the reduction of reported false-positive or false-negative mutant events.

P32

Integration of *cII* and *Pig-a* Mutation and Micronucleus Endpoints into the Big Blue® Transgenic Rat Mutation Assay: Results for Benzo(a)pyrene (BaP) and *N*-Ethyl-*N*-nitrosourea (ENU). Young RR[1], Dinesdurage H[1], Elbekai RH[1], Bruning D[1], Lawlor TE[1], LeBaron MJ[2], Ji Z[2], Sosinski LK[2], Aardema MJ[1,3]. [1]BioReliance by SAFC, Rockville, MD, United States, [2]Dow Chemical Company, Midland, MI, United States, [3]Marilyn Aardema Consulting, LLC, Fairfield, MI, United States.

Recent guidelines (OECD TG488 and ICH S2R1) have led to renewed interest in transgenic rodent mutation assays and integration of multiple endpoints into repeat dose studies. We evaluated male Big Blue® Fisher 344 rats for induction of *cII* mutations in liver and bone marrow, *Pig-a* mutant phenotype reticulocytes (RET^{CD59-}) and erythrocytes (RBC^{CD59-}) and micronucleated reticulocytes (mnRET) and erythrocytes (mnNCE). Treatments were with olive oil (5 mL/kg/day) or BaP (50 mg/kg/day) for 28 days or ENU (20 mg/kg/day) on Days 1-3. Five animals/group were bled (Day 29) and tissues collected (Day 31). Significant increases ($p<0.001$) in *cII* mutant frequencies were observed for BaP in liver (5.8-fold) and bone marrow (9.6-fold), and ENU in liver (3.6-fold) and bone marrow (6.8-fold). Blood was analyzed for *Pig-a* mutant phenotype and micronuclei by flow cytometry using MutaFlow® and MicroFlow® kits, respectively (Litron). BaP induced significant increases ($p<0.05$) in RET^{CD59-}, RBC^{CD59-}, and mnRET (61.1-, 12.2-, and 3.8-fold, respectively). ENU induced significant increases ($p<0.001$) in RET^{CD59-} and RBC^{CD59-} (156.1- and 53.6-fold, respectively). As expected, due to the 25 day interval between ENU dosing and blood sample collection, increases were not observed in mnRET or mnNCE with ENU. The lack of increase in mnNCE for BaP is likely due to splenic filtration of micronucleated RBCs in rats. Overall, the Big Blue® F344 Rat Mutation Assay can robustly detect mutagenesis in somatic tissues by direct and indirect acting mutagens, and *Pig-a* mutation and micronuclei measurements can easily be integrated. Attention to timing between dosing and blood collection is important for optimal micronuclei detection.

P33

Evaluation and Comparison of Big Blue® Mouse and Rat Transgenic Rodent (TGR) Mutation Assays with *N*-Ethyl-*N*-Nitrosourea (ENU) And Benzo(a)pyrene (BaP). Young RR[1], Dinesdurage H[1], McKeon ME[1], Elbekai RH[1], Bruning D[1], Lawlor TE[1], Aardema MJ[1,2]. [1]BioReliance by SAFC, Rockville, MD, United States, [2]Marilyn Aardema Consulting, LLC, Fairfield, OH, United States.

Finalization of OECD TG 488 resulted in increased interest and use of transgenic rodent mutation assays. One advantage of the Big Blue® TGR assay is availability in two species, either mouse (C57BL/6 or B6C3F1) or rat (Fisher 344). We qualified both species to new OECD TG 488 standards and to permit comparisons between the two species. Homozygous male Big Blue® C57BL/6 mice and F344 rats received olive oil vehicle (5 mL/kg/day) or BaP (50 mg/kg/day) for 28 days or ENU (40 mg/kg/day for mice or 20 mg/kg/day for rats on Days 1-3) with necropsy on Day 31. Mutations in liver and bone marrow were evaluated. Background mutant frequency in each tissue was similar in mice and rats. Liver background mutant frequency was $43.6\pm6.2\times10^{-6}$ in mice and $43.4\pm8.8\times10^{-6}$ in rats. Bone marrow background mutant frequency was $38.1\pm14.2\times10^{-6}$ in mice and $29.7\pm14.3\times10^{-6}$ in rats. In mice, significant ($p<0.001$) increases in mutant frequencies were observed with BaP in liver (4.9-fold) and bone marrow (17.7-fold) and ENU in liver (4.2-fold) and bone marrow (10.6-fold). In rats, significant ($p<0.001$) increases in mutant frequencies were observed with BaP in liver (5.8-fold) and bone marrow (9.6-fold) and ENU in liver (3.6-fold) and bone marrow (6.8-fold). Both Big Blue® mouse and rat systems are robust with the ability to detect significant increases in mutagenicity in slow dividing (liver) and fast dividing (bone marrow) tissues by a direct acting mutagen and one that requires metabolic activation. Response was similar to ENU and BaP in both mice and rats.

P34

Integration of Multiple Endpoints into the Big Blue® Transgenic Mouse Mutation Assay: Assessment of *cII* and *Pig-a* Mutation and Micronucleus Induction by Benzo(a)pyrene and *N*-Ethyl-*N*-nitrosourea. Young RR[1], Dinesdurage H[1], Stankowski LF[1], Kulkarni R[1], Lawlor TE[1], McKeon M[1], Xu Y[1], Bruning D[1], Avlasevich S[2], Torous DK[2], Dertinger SD[2], Aardema MJ[1,3]. [1]BioReliance by SAFC, Rockville, MD, United States, [2]Litron Laboratories, Rochester, NY, United States, [3]Marilyn Aardema Consulting, LLC, Fairfield, OH, United States.

Integration of multiple genotoxicity endpoints into repeat-dose rodent studies provides broad assessment of genotoxic potential of different modes of action while reducing the use of animals. Using homozygous male Big Blue® C57BL/6 mice, we examined the induction of *cII* mutations in liver and bone marrow, *Pig-a* mutant phenotype reticulocytes ($RETCD^{24-}$) and erythrocytes ($RBCCD^{24-}$), and micronuclei in blood reticulocytes (mnRET) and mature erythrocytes (mnNCE). Animals were treated with olive oil (5 mL/kg/day for 28 days), BaP (50 mg/kg/day for 28 days), or ENU (40 mg/kg/day on Days 1, 2 and 3). Blood and tissues were collected on Day 31. Significant increases ($p<0.001$) in *cII* mutant frequencies were observed for BaP in liver (4.89-fold) and bone marrow (4.15-fold), and for ENU in liver (17.7-fold) and bone marrow (10.6-fold). Peripheral blood was collected ~3 hours before sacrifice and analyzed for *Pig-a* mutant phenotype and micronucleus frequencies by flow cytometry using MutaFlow® and MicroFlow® kits, respectively (Litron Laboratories). BaP induced significant increases ($p<0.001$) in $RETCD^{24-}$, $RBCCD^{24-}$, mnRET, and mnNCE (616-, 101-, 1.56-, and 2.11-fold, respectively). ENU induced significant increases ($p<0.001$) in $RETCD^{24-}$, $RBCCD^{24-}$, and mnNCE (1050-, 208-, and 1.40-fold respectively). As expected, mnRET were not elevated by ENU ($p>0.05$) due to the interval between last dose administration and blood collection. This study demonstrates the robustness of the Big Blue® Mouse Mutation Assay to detect induced mutagenesis by both a direct acting mutagen and one requiring metabolic activation. This work also demonstrated the ability to integrate additional endpoints for mutation and clastogenicity.

P35

Delayed Effects of a Whole-Body Exposure to Low-Dose Radiation on Somatic and Germinal Cells of Mice. Jangiam W, Gordon C, Rithidech KN. Department of Pathology, Stony Brook University, Stony Brook, NY, United States.

In spite of extensive research, assessment of potential health risks associated with exposure to low-dose radiation at doses less than or equal to 0.05 Gy/year (the existing limit for exposure in the workplace) of radiation is still a challenging public-health issue. In this study, we determined the delayed effects of low doses (0.05 or 0.1 Gy) of ^{137}Cs γ rays on somatic (bone-marrow and lung) and germinal (testis) cells of BALB/cJ mice collected at 6 months post-irradiation. Mice exposed to 0 or 1 Gy of radiation served as sham- or positive-controls, respectively. Three biological-endpoints were used to determine the effects of low-dose radiation: 1) apoptosis, 2) inflammatory responses, and 3) DNA methylation: 5-methyl-cytosine and 5-hydroxymethyl-cytosine. We found no changes in the levels of any of the studied biological endpoints in the bone-marrow, lung, or testis of mice exposed to a single dose of 0.05 Gy of radiation, in relation to those in sham controls. However, a single dose of 0.1 or 1 Gy of radiation induced significant increases in the levels of apoptosis and activated NF-kB and pro-inflammatory cytokines in all tissues selected for study. A significant reduction in the levels of 5-hydroxymethyl-cytosine was detected in the tissues collected from mice exposed to 0.1 or 1 Gy (but not 0.05 Gy) of radiation. Overall, our new data suggest that low-dose radiation (as low as 0.05 Gy) is incapable of inducing delayed apoptosis, inflammation, and aberrant-patterns of DNA methylation in both somatic and germinal cells of exposed mice. Research funded by NASA Grant# NNX11AK91G.

P36

Red Raspberries in the Maternal Diet Alter PAH Induced Gene Expression in Offspring of a Transplacental Mouse Model. Bunde KB, Siddens LK, Larkin A, Krueger SK, Williams DE, Baird WM. Oregon State University, Corvallis, OR, United States.

Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental carcinogens found in cigarettes, food, and byproducts of carbon based fuel production and combustion. While not all PAHs are carcinogenic, dibenzo[*def*,*p*]chrysene (DBC) and benzo[*a*]pyrene (B[*a*]P) are two PAHs used in animal models and are suspected and known human carcinogens, respectively. PAHs are bioactivated through several known metabolic pathways; the most well studied involves the Cytochrome (CYP) P450 CYP1 family of enzymes regulated by the aryl hydrocarbon receptor. Dietary PAH exposure, either from PAHs originating from food sources or introduced during high temperature cooking, accounts for a significant portion of total PAH burden in non-smokers. Chemoprevention through co-exposure from dietary agents, such as anthocyanins, can alter metabolic pathways and potentially reduce the risk of carcinogenesis. Fruits and berries in particular contain significant concentrations of anthocyanins whose chemopreventative potential was investigated in this study using a transplacental mouse model. Pregnant mice were fed either AIN93G (control) diet or diet with 5% freeze-dried red raspberries from gestation day (GD) 9 up to the end of lactation. On GD 17, dams were gavaged with either corn oil, DBC, or B[*a*]P. A subsample of pups were harvested 24 hours post gavage and lung RNA was analyzed for changes in gene expression. TNF upregulation by DBC and downregulation of Timp1 by DBC and B[*a*]P demonstrates the PAHs tumorigenic potential. Downregulation of TNF and upregulation of Timp1 by raspberries suggests consumption of raspberries could help protect against tumor metastasis and decrease the inflammatory response.

P37

Does Diet-Induced Obesity Act as a Weak Mutagen?. Wilson MJ[1], Simon BR[1], Dertinger SD[2], Torous DK[2], Avlasevich SL[2], Wickliffe JK[1]. [1]Tulane University, New Orleans, LA, United States, [2]Litron Laboratories, Rochester, NY, United States.

Obesity increases the risk of a number of chronic diseases in humans including several cancers. Biological mechanisms responsible for such increased risks are not well understood at present. Increases in systemic inflammation and oxidative stress, endogenous production of mutagenic metabolites through microbiological processes or excess calorie and fat metabolism, altered signaling in proliferative or antiproliferative pathways, and increased sensitivity to exogenous mutagens and carcinogens are likely at play. Therefore, we hypothesize that obesity alone creates an endogenously mutagenic environment in addition to increasing the sensitivity to environmental mutagens. To initially test part of our hypothesis, we examined somatic mutation frequencies in a mouse model of diet-induced obesity. Mutation frequencies in the Pig-a reporter gene and micronucleus frequencies were determined in blood cells (erythrocytes and reticulocytes) in 30-week old male mice reared on a high-fat diet (60% calories from fat) that exhibit an obese phenotype and matched mice reared on a normal-fat diet (10% calories from fat) that do not exhibit an obese phenotype. N-ethyl-N-nitrosourea (ENU) was used a positive mutation control. ENU induced a robust mutation response in both endpoints with no differences between phenotypes. Micronucleus frequencies were not significantly different between untreated mice of both phenotypes. However, untreated, obese mice had a significantly higher Pig-a gene mutation frequency (2.5-fold, $p < 0.03$) than untreated nonobese mice. This suggests that obesity alone in the absence of an exposure to an exogenous mutagen is itself mutagenic, and these initial results support part of our working hypothesis.

P38

Induction of CNVs in the F1 Generation of Individuals Accidentally Exposed to Ionizing Radiation from Cesium-137 Reviewed by CMA (Chromosomal Microarray). Costa EOA[1], Pinto IP[2], Franco FC[3], da Silva JF[2], Godoy FR[3], Melo COA[3], Silva DM[3], da Silva CC[2], da Cruz AD[2], Pereira RW[1]. [1]Universidade Católica de Brasília, Brasília, Distrito Federal, Brazil, [2]Pontifícia Universidade Católica de Goiás, Goiânia, Goiás, Brazil, [3]Universidade Federal de Goiás, Goiânia, Goiás, Brazil.

The radiological accident in Goiânia in 1987, which occurred in the Brazilian Highlands, resulted in a severe episode of human contamination. Cell exposure to ionizing radiation induces several damages in nucleic acids, especially on DNA. Currently established that chromosomal microarray analysis (CMA), molecular cytogenetics technique is an important tool for the detection of copy number variations (CNVs) (deletions or duplications) on the human genome. During cell division, CNVs may implicate on DNA replication errors, some studies have reported that many agents or conditions, which lead to replication stress, have the potential to induce CNV. In this study, we determined the frequency of CNVs in the F1 generation of individuals exposed to ionizing radiation from Cesium-137 during the accident in Goiânia, Goiás, Brazil. The exposed group consisted of 6 families, of which at least one parent was directly exposed to ionizing radiation from Cesium-137(absorbed dose ≤ 0.2 Gy). The control group were not exposed to ionizing radiation. Kolmogorov-Smirnov statistical test compared the frequencies of CNVs, and showed no statistically significant differences between the frequencies of CNVs in the treatment group and the control group ($p = 0.84$). Thus, a possible reason for a small rate of CNVs observed in this study may be related to low doses estimated absorbed radiation to the exposed population that compound the study group. On the other hand, exposure to discrete biological doses of ionizing radiation systems seem to stimulate DNA repair system, which behaves more efficiently.

P39

Profile of Select Hepatic Insulin Signaling Pathway Genes in Response to 2-Aminoanthracene Dietary Ingestion. Mattis ND[1], Jay JW[1], Barnett BW[1], Rosaldo JJ[1], Howerth EW[2], Means JC[3], Gato WE[1]. [1]Georgia Southern University, Department of Chemistry, Statesboro, GA, United States, [2]Department of Pathology, College of Veterinary Medicine, The University of Georgia, Athens, GA, United States, [3]Bren School of Environmental Science & Management, University of California, Santa Barbara, Santa Barbara, CA, United States.

Some genes that regulate various processes such as insulin signaling, glucose metabolism, fatty acid and lipid biosynthesis were profiled. The objective of the current investigation is to examine the mRNA expression of some genes that mediate insulin signaling due to 2AA toxicity. 2AA is a polycyclic aromatic hydrocarbon (PAHs) that has been detected in broiled food and tobacco smoke. Twenty four post-weaning 3-4 weeks old F344 male rats were exposed to 0mg/kg-diet, 50mg/kg-diet, 75mg/kg-diet and 100mg/kg-diet 2AA for 2-weeks and 4-weeks. The mRNA expression of akt1, g6pc, gck, glut4, insr, irs1, pp1r3c, pampk, socs2, and srebf1 was determined by qRTPCR followed by the quantification of g6pc and ampk via ELISA. Results seem to suggest 2AA modulates these genes depending on the length of exposure. Up-regulation of ampk and socs2 genes in animals treated with 100mg/kg-diet and 50mg/kg-diet respectively during 14 days of feeding was noted. G6pc expression seems to be inhibited in the 2-weeks group while dose-dependently increased in the 4-weeks group. Hepatic activity of g6pc was enhanced significantly in the livers of rats that ingested 2AA. It appears 2AA intoxication leads to the activation of irs1 and akt1 genes in the liver. Quantified ampk amounts significantly increased in the short-term treatment group. Dose-dependent rise of ampk in animals treated to 2AA suggest an increase production of hepatic ampk in response to the toxicity of 2AA in order to maintain cellular homeostasis. In contrast, the reduction in ampk concentration in treated animals within the 4-week set indicated an adaptive recovery.

P40

Total Intravenous Anesthesia May Alter Gene Expression in Patients Undergoing Elective Surgery. Braz MG[1], Evangelista AF[2], Silva GN[3], Braz JR[1], Braz LG[1], Salvadori DM[1]. [1]Sao Paulo State University (UNESP), Botucatu Medical School, Botucatu, Sao Paulo, Brazil, [2]Molecular Oncology Research Center, Barretos Cancer Hospital, Barretos, Sao Paulo, Brazil, [3]Federal University of Ouro Preto (UFOP), Ouro Preto, Minas Gerais, Brazil.

Although general anesthesia is routinely used as an essential surgical procedure, little is known about its comprehensive influence that is not reflected in mortality and morbidity. There is no report in literature about gene expression profiling in patients undergoing total intravenous anesthesia (TIVA). Therefore, the current study aimed to evaluate the effects of TIVA with propofol on gene expression profiling in peripheral blood cells from patients undergoing elective surgery. The Ethical Committee approved the protocol, which included 25 adult patients of both genders, classified by the American Society of Anesthesiologists (ASA) as physical status I (healthy patient with no disease other than a surgical abnormality), and who were scheduled for minimally invasive otorhinological surgery. Blood samples were collected before anesthesia (baseline) and 120 min after the beginning of propofol anesthesia. RNA was isolated from whole blood, RNA quality was assessed and Whole Human One-Color Agilent 4x44K microarray was used. Data analysis was performed in R statistical environment and MeV software. Data pre-processing, quantile normalization and paired t-test with Bonferroni correction ($p < 0.05$) were applied. Results showed that a few genes were differentially expressed during propofol anesthesia. Most of the genes were downregulated at 120 min after the beginning of propofol anesthesia, but genes such as *ING4* and *GCC2* were upregulated, and were validated by real time PCR. These findings suggest that propofol anesthesia can modulate gene mostly related to the regulation of cell growth and signal transduction pathways, cellular functions, cell death, and DNA repair.

P41

Building Predictive Gene Signatures through Simultaneous Assessment of Transcription Factor Activation and Gene Expression. VanDuyn NM, Franzosa JA, Houck KA, Ward WO, Chorley BN, Corton JC. US Environmental Protection Agency, NHEERL, Research Triangle Park, NC, United States.

Many drugs and environmentally-relevant chemicals can cause adverse outcomes in the rodent liver. Adverse outcomes, such as cancer, are linked to molecular initiating events (MIE) and downstream key events to define adverse outcome pathways (AOP). Identification of gene sets (signatures) that are predictive of either MIEs or key events would be useful in predicting AOP modulation after chemical exposure in high-throughput screening. This project seeks to identify signature genes for transcription factor (TF) activation via simultaneous assessment of TF activity and global gene expression. RNA from HepG2 cells exposed in concentration-response to ToxCast or reference chemicals was used to assess TF activation at over 50 cis-elements with Attagene FACTORIAL assays and expression of more than 47,000 RNA targets using the Illumina HumanHT-12 Expression BeadChip. Genes were identified which exhibited expression changes that correlated with activation of one or more TF. ToxCast curve fitting algorithms were applied to the gene expression data to identify genes that exhibit a significant concentration-response and signatures for AhR, TRalpha, and PPARgamma were developed. Using the Running Fishers test, derived signatures were queried against the NextBio database to find biosets, which exhibited a significant positive correlation to the signature. The AhR signature identified biosets associated with known AhR-activating chemicals including TCDD, benzo(a)pyrene and quercetin ($p\text{-value} < 1x10^{-33}$), thus validating the method. Future work will expand the analysis to other TFs, allowing a comprehensive assessment of chemical-induced modulation of multiple human TFs in large, publically available, genomic datasets. This abstract does not represent EPA policy.

P42

***APOE* Genotype Is Associated to Decreased Risk for Coronary Artery Disease.** Padovani JL[1], Bazo AP[1], da Silva GN[2], de Camargo EA[1], Salvadori Jr D[3], Salvadori RAF[4], Padovani CR[5]. [1]Botucatu Medical School, São Paulo State University, Botucatu, São Paulo, Brazil, [2]School of Pharmacy, Universidade Federal de Ouro Preto, Botucatu, São Paulo, Brazil, [3]Beneficência Portuguesa Hospital, São Paulo, São Paulo, Brazil, [4]São Luiz Hospital, São Paulo, São Paulo, Brazil, [5]Biosciences Institute of Botucatu, São Paulo State University, Botucatu, São Paulo, Brazil.

Coronary artery disease (CAD) remains as one of the main world health problems, and the *apolipoprotein E* (*ApoE*) gene have been one of the targets of research interest. This gene has three polymorphisms (*e*2, *e*3 and *e*4) encoding three isoforms of the protein (ApoE2, E3 and E4, respectively). ApoE is synthesized mainly in the liver and plays an important role in metabolism of cholesterol and triglycerides, as well as for stabilizing lipoproteins in the blood. The present study aimed to investigate the relationship between *APOE* gene polymorphisms and susceptibility for CAD. A case-control study was conducted with 156 subjects with CAD (at least with one coronary artery obstructive lesions ≥ 20%) and 151 control subjects (with < 20% coronary artery obstruction). Genetic polymorphisms were identified by RFLP-PCR (polymerase chain reaction restriction fragment length polymorphism). First of all, data confirmed the positive correlation between demographic and medical findings (age, sex, hypertriglyceridemia, hypertension and diabetes) and the CAD diagnostic. The frequency of *E3E4* genotype was significantly lower in CAD patients (26,3%) than in controls (41,7%). Therefore, the results provided evidence the *APOE* genotype is associated with a decreased risk for coronary artery disease. Financial Support: FAPESP and CNPq.

P43

Whole Genome Gene Expression Profiling of Cardiac Tissue from Mice Exposed to Doxorubicin. Fuscoe JC, Vijay V, Han T, Desai VG. US Food and Drug Administration, NCTR, Jefferson, AR, United States.

Doxorubicin (DOX) is an effective anticancer drug whose use is limited by a dose-dependent risk of cardiotoxicity leading to heart failure. Current biomarkers of cardiotoxicity, such as cardiac troponins, are released into the plasma only after cardiac cell injury. There is a need for biomarkers that can accurately predict risk of cardiac injury before it occurs. A newly developed mouse model of DOX-induced cardiotoxicity was used to examine gene expression changes in the heart. B6C3F1 mice were given weekly intravenous injections of 3 mg/kg DOX or saline for 2, 3, 4, 6, and 8 weeks, resulting in cumulative DOX doses of 6, 9, 12, 18, and 24 mg/kg. Tissue injury, as evidenced by increased cardiac troponin, occurred at doses above 18 mg/kg and cardiac lesions were observed at 24 mg/kg. RNA was isolated from cardiac tissue and gene expression profiling was conducted. The expression of thousands of genes was changed ($p<0.05$ and fold-change >1.5) in DOX-treated mice compared to concurrent saline controls, with most changes resulting in increased expression. 112 transcripts were significantly ($p<0.05$) up-regulated at least 1.5-fold at all cumulative doses. This gene set was enriched for functions related to cell cycle control and DNA repair. Included in this set was the anti-tumorigenic target of DOX, topoisomerase 2, suggesting compensation for inhibition of this critical enzyme. In addition, hundreds of genes were uniquely differentially expressed at each cumulative dose, indicating the complex biological response in the heart to this dosing regimen that is similar to what is used clinically.

P44

Transcriptional Alterations in the SOD2 Gene Is Not Related to the Newborn Obesity Predisposition. Marcondes JPC[1], Silveira MAD[1], Prado RP[1], Andrade PFB[1], Luperini BCO[1], Rudge MVC[2], Salvadori DMF[1]. [1]Department of Pathology, Botucatu Medical School, São Paulo State University, UNESP, Botucatu, São Paulo, Brazil, [2]Department of Gynecology and Obstetrics, Botucatu Medical School, São Paulo State University, Botucatu, São Paulo, Brazil.

Obesity is a multifactorial disease, associated with a range of metabolic disturbances. Environmental factors contribute for BMI (Body Mass Index) variation, however, the genetic background can be also responsible. Several findings have demonstrated that *in utero* stressors (such as gestational diabetes, nutritional alteration, etc.) can promote transcriptional and posttranscriptional changes, besides affecting mitochondrial metabolism, which could be involved in the etiology of diabetes, obesity, and their comorbities. Furthermore, it is known that epigenetic events can also modulate fetal development and predispose to later development of diseases, including obesity and other metabolic syndromes. Therefore, this study aimed to identify, in cells from placenta (fetal and maternal sides) and umbilical cord blood of newborns from mothers with gestational diabetes mellitus (GDM), whether transcriptional alterations in the SOD2 gene (gene expression), involved in mitochondrial metabolism, are related to an increased risk for developing obesity in adulthood. The study included pregnant women with GDM (n = 10) and their respective newborns (n = 10), and healthy pregnant women (n = 10) and their newborns (n = 10). Additionally, adult obese (n = 10) and eutrophic (n = 10) subjects were also included as reference populations. Results demonstrated no alterations in SOD2 gene expression among the groups. Thus, this preliminary data showed no modulation promoted by GDM and/or obesity on transcriptional levels of SOD2 gene. Supported by FAPESP and CNPq.

P45

High-Resolution Genotyping Arrays Document Genetic Variation at Nonsynonymous SNPs Providing Insight into Environmentally-Responsive Genes. Milojevic M, Charron B, Edge AK, Eitutis ST, Hill KA. Western University, London, ON, Canada.

The extent and nature of environmental influences on the landscape of mutations across a genome are poorly understood. High-resolution SNP genotyping arrays with a large number of nonsynonymous SNPs offer a sensitive biomonitoring tool to detect adaptive phenotypes under strong positive selective pressures in different environments. We hypothesized that different positive selective pressures for wild-caught and laboratory-bred mice would be reflected in the nature of the genes impacted by nonsynonymous SNP differences. Genotyping data at 2,362 nonsynonymous SNP loci were obtained using publically available Mouse Diversity Genotyping Array (MDGA) data files for 19 wild-caught, 50 wild-derived and 116 classical laboratory mice. The function of genes with nonsynonymous SNP variation was determined using IPA and DAVID. Gene lists for the three mouse groups were compared to each other and to the entire list of genes with nonsynonymous SNPs. Gene interaction networks in the three mouse groups differed from the gene networks in the entire nonsysnonymous SNP list. For the entire nonsysnonymous SNP list, the highest ranked network term was cell cycle. The top gene interaction networks differed for each mouse group although cancer, metabolism and cell-related terms were common to all three groups. The most highly ranked network in wild-caught mice, lipid metabolism, ranked lower in wild-derived mice and was absent in classical laboratory mice. Lipid metabolism genes were previously found to be overrepresented in wild-caught mice in copy number variant regions (Henrichsen et al., 2009, *Nat Genet*, 41:424). The MDGA offers low cost detection of environmentally-responsive genes.

P46

Micronutrient Supplementation and Genetic Damage in Obese Brazilian Women. Luperini Bruno CO[1], Campos Joara P[1], Marcondes Joao Paulo C[1], Prado Renato P[1], Novais Patricia SF[1], Oliveira Maria Rita M[2], Salvadori Daisy MF[1]. [1]Botucatu Medical School, UNESP, São Paulo State University, Botucatu, Sao Paulo, Brazil, [2]Bioscience Institute, UNESP, São Paulo State University, Botucatu, Sao Paulo, Brazil.

Obesity is a multifactorial disease including complexes interactions between genetic and environmental factors, and it is associated with increased risk for metabolic syndrome, type 2 diabetes mellitus, and heart diseases. This study aimed to assess the relationship between micronutrient intake, genetic damage and cytokines blood concentration in morbid obese women (n = 30; BMI = 45.89 ± 6.8) compared to healthy eutrophic women (BMI = 21.56 ± 1.55). The supplementation consisted of two DRI (Dietary References Intakes) per day, during 8 weeks before and 24 weeks after bariatric surgery. Obese women presented improvement of folic acid (7.73±2.0 versus 13.9 ± 6.98), vitamin E (14.0 ±3.22 versus 16.84±3.65) and B12 (226.04±79.67 versus 320.96±271.2) 24 weeks after bariatric surgery. No difference was detected for other micronutrients such as vitamins A and C, selenium, iron and zinc after bariatric surgery. The comet and micronucleus assays were used to assess, respectively, DNA and cytogenetic damage in lymphocytes. Obese women presented significantly higher amount of DNA damage (53.40 ± 22.19) than the eutrophic (22.2 ± 19.7) subjects. However, after micronutrient supplementation there was a decrease of DNA damage ($p < 0.05$) in obese women, but the amount continued different from control. Same result was observed in the micronucleus test. Significant ($p < 0.05$) decrease of cytokines (IFN-γ, IL-8, e TNF-α) was detected in obese women after surgery and micronutrients supplementation. In conclusion, our data demonstrated that micronutrient supplementation may decrease genetic damage in morbid obese women. Financial Support: FAPESP and CNPq (Brazil).

P47

Bromodeoxyuridine Treatment to Measure Hepatocellular Proliferation Does Not Mask Furan-Induced Gene Expression Changes in Mouse Liver. Webster AF[1,2], Williams A[1], Recio L[3], Yauk CL[1]. [1]Health Canada, Ottawa, ON, Canada, [2]Carleton University, Ottawa, ON, Canada, [3]ILS, Research Triangle Park, NC, United States.

Bromodeoxyuridine (BrdU) is a synthetic nucleoside used to detect cellular proliferation. BrdU incorporates in the place of thymine but pairs with guanine, thereby increasing the risk of transition mutations in dividing cells. Thus, standard practice is to use a second cohort of animals for parallel toxicogenomics studies; however, the impact of BrdU on global gene expression is unknown. To test whether this second cohort of animals is actually required we performed transcriptional profiling to determine if the molecular mode of action (MOA) of furan, a liver carcinogen, could be detected in BrdU-treated samples. Global hepatic gene expression was measured using Agilent microarrays in mice that were sub-chronically exposed to furan in the presence (+BrdU) or absence (-BrdU) of BrdU. Exposure to BrdU resulted in minimal gene expression changes. A comparison of +BrdU versus -BrdU control mice revealed only 11 differentially expressed probes (DEPs); this number fell to 3 in the presence of a carcinogenic dose of furan. Next, DEP lists generated for furan-treated versus control -BrdU mice were compared to DEP lists for +BrdU mice. Some differences were observed in the +BrdU samples; however, they did not affect hierarchical clustering, nor did they impair detection of the furan MOA. Taken together, we propose that BrdU-treated mice could be used for toxicogenomic analyses, which would halve the number of rodents required. However, we recommend that further validating experiments be performed with additional chemicals operating through other modes of action before the use of BrdU-treated animals in 'omics studies becomes common practice.

P48

A Testing Strategy for Genotoxic Impurities Which Are Potential Metabolites. Zeller A, Kirchner S, Muster W, Paehler A, Rothfuss A, Schadt S, Singer T. Roche, Basel, Switzerland.

Arylamines (AA) are convenient building blocks which form chemical bonds under mild reaction conditions. They are part of many drug molecules, but some members of this chemical class are known mutagens or carcinogens. Arylamines can be liberated metabolically or via non-enzymatic hydrolysis. As a consequence, arylamines may be contained in drug products as impurities and/or appear as metabolites after drug intake. Mutagenicity assessment for potentially genotoxic impurities is a regulatory requirement. DNA-reactive mutagenic impurities should be either avoided or analytically controlled to a regulatory acceptable level, which is 1.5 µg/d for lifelong exposure - according to the Threshold of Toxicological Concern (TTC) concept. Such approaches, however, cannot be applied to mutagenic impurities which are also formed as intermediates during metabolism, because analytical proof of *in vivo* absence of a metabolite is not possible with current technology. We developed a testing strategy that combines *in silico* and *in vitro* assessments of mutagenicity with a dedicated database to avoid unnecessary testing and continuously improves the predictivity of our *in silico* tools. It takes plausibility of metabolic formation and coverage in existing assays like standard mutagenicity screening into account. This approach maintains a high sensitivity for the detection of potentially carcinogenic aromatic amines (> 85% for a representative chemical space), while balancing the demands of medicinal chemists and toxicologists with project timelines. Ultimately, it avoids regulatory issues and safety concerns associated with the undue exposure of patients to mutagenic and possibly carcinogenic by-products, degradants, or metabolites.

P49

Considering Molecular Mechanism and Other Parameters While Constructing Expert Analyses of ICH M7 (Q)SAR Predictions of Bacterial Mutagenesis. Benz RD[1], Myatt GJ[2], Cross KP[2]. [1]OmnyCorp, Rockville, MD, United States, [2]Leadscope, Inc., Columbus, OH, United States.

The new International Conference on Harmonisation (ICH) Guideline M7, *Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk*, states that "The outcome of any computer system-based analysis should be reviewed with use of expert knowledge in order to provide additional supportive evidence on the relevance of any positive or negative prediction..." This poster suggests an outline for standardizing ICH M7-related submissions to regulatory agencies on predicted genotoxic impurity mutagenicity, focusing on the factors considered during human expert analysis of computer predictions. In general, submissions to regulatory agencies should include: 1) the name and version number of the (Q)SAR software program(s) used; 2) the results of known laboratory experiments and software predictions, including actual bacterial G:C and A:T mutagenesis testing data; 3) statistical predictions by (Q)SAR software of G:C and A:T mutagenesis; 4) expert alert predictions of bacterial mutagenesis; 5) an expert analysis; and 6) an overall conclusion. This presentation explores and builds upon current discussions of such details of the expert analysis as: 1) an assessment of available laboratory data; 2) relevant information available from proprietary knowledge; 3) the results of read-across or chemical category assessment; 4) a consideration of the environment of structural alerts within the impurity; 5) an examination of the physical-chemical properties of the impurity; 6) the reliability of alert and/or model features; and 7) a consideration of the mechanistic plausibility of an alert being a mutagen. A case study is presented illustrating construction of an expert opinion for a submission.

P50

Revision of OECD Guidelines for Genotoxicity Testing: Current Status and Next Steps. Schoeny RS[1], Thybaud V[2], van Benthem J[3], Delrue N[4], Douglas GR[5], Lorge E[6], Lovell DP[7], Singer T[5]. [1]US Environmental Protection Agency, Washington, DC, United States, [2]Sanofi, Vitry-sur-Seine, France, [3]National Institute for Public Health and the Environment, Bilthoven, Netherlands, [4]OECD, Paris, France, [5]Health Canada, Ottawa, ON, Canada, [6]Servier Group, Orleans-Gidy, France, [7]St George's, University of London, London, United Kingdom, [8]ENVIRON International Corporation, Little Rock, AR, United States.

Over the past 30 years, assays have been developed to evaluate chemical genotoxicity. OECD Genotoxicity Test Guidelines (TG) describe assay procedures for regulatory safety testing. Since the last OECD TG revision (1997), there has been tremendous scientific and technological progress on assay performance and understanding of underlying mechanisms. To improve regulatory assay performance and data interpretation, OECD began updating TGs in 2010. OECD identified Canada, Netherlands, France and USA as lead countries and established an international Expert Group (EG). The EG recommended deletion of several TGs, including assays in yeast and *Drosophila*, as mammalian cell assays are considered more relevant. TG were revised to 1) improve clarity and consistency; 2) improve statistical power; 3) refine methodology to improve relevance of results; and 4) provide up-to-date recommendations for acceptability criteria and data interpretation. In April 2014 the OECD Working group of National Coordinators of the Test Guidelines Programme approved revised TGs for *in vitro* and *in vivo* micronucleus (TG487, TG474), and chromosomal aberration tests (TG473, TG475), and a new TG for *in vivo* Comet assay. Next steps are these: 1) revision of TGs for *in vivo* germ cell and *in vitro* mammalian gene mutation tests; 2) updating the Introduction Document, providing a TG overview and background for the recent updates; 3) a Guidance Document on assay strengths and weaknesses, weight of evidence approaches, and data interpretation; 4) a new TG on *in vitro* TK mutation assay. The opinions are the authors and do not necessarily reflect policies of US EPA.

P51

A Systematic Approach to Organizing Mechanistic Data for Risk Assessment by Linking Endpoints with Informative Mechanistic Characteristics of Carcinogenesis. Gibbons CF[1], Caldwell JC[1], Chiu WA[1], DeMarini DM[2], Fritz JM[1]. [1]National Center for Environmental Assessment, Office of Research and Development, US EPA, Washington, DC, United States, [2]National Health and Environmental Effects Research Laboratory, Office of Research and Development, US EPA, Research Triangle Park, NC, United States.

The assessment of carcinogenic hazards by organizations such as IARC and EPA's IRIS (Integrated Risk Information System) Program requires collating and analyzing mechanistic data to inform conclusions about tumor-site concordance and coherence (IARC), biological relevance of non-human data, susceptible subpopulations or life stages, and low-dose-response relationships (EPA). The National Research Council has emphasized the need for consistent, transparent, and systematic approaches for the identification, evaluation, and integration of data for assessing hazards to human health. Mechanistic studies present a challenge because they are typically both numerous and diverse and involve a multitude of targets and toxicity pathways. In order to capture and analyze study findings in a systematic way, we propose organizing endpoints and relevant assays according to a list of characteristics derived from the current understanding of carcinogenesis. For example, endpoints from a study reporting comet assay results and cell survival would be categorized under "DNA damage" and "changes in cell death and division," respectively. Such a categorization would serve as a guide in the first step in the systematic analysis of mechanistic data. The proposed categories are not mutually exclusive and are not themselves "modes of action" but rather more accurately reflect the mechanistic database and what is currently understood regarding carcinogenesis. As a case study, we applied our approach to the large mechanistic database for hexavalent chromium. Data organized in such a way may facilitate generation and analysis of hypothesized modes of action and toxicity pathways. This abstract does not reflect US EPA policy.

P52

Chemically-Induced Mouse Lung Tumors: Applications to Human Health Assessments. Keshava N, Woodall G, Keshava C, Reinhart P. National Center for Environmental Assessment, Office of Research and Development, US EPA, Research Triangle Park, NC, United States.

A state-of-the-science workshop on chemically-induced mouse lung tumors was conducted by US Environmental Protection Agency to discuss issues related to the use of mouse lung tumor data in human health assessments. Naphthalene, styrene, and ethylbenzene were chosen for the analysis due to the commonality of mouse lung tumors in all these three environmental chemicals. The goals of the workshop were to identify the evidence, from multiple scientific disciplines, regarding formation of chemically-induced lung tumors in mice; discuss analysis and interpretation of the evidence; discuss how such evidence informs human health assessments; and identify commonalities, linkages, or differences between the evidence from various disciplines and across the chemicals. Evidence informing the association between occupational exposure to styrene, ethylbenzene, or naphthalene and lung cancer; comparative biology of mouse lung tumors, associated pathologic effects, issues related to tissue and species concordance; mode of action analysis and biological mechanisms including pharmacokinetics and pharmacodynamics; and evidence from cellular, genetic and molecular toxicity was discussed. In summary, although consensus was not sought, the panelists agreed that data showing mouse lung tumors with chemical exposures can be relevant for human health risk evaluation on an individual chemical basis. Key data gaps were identified that would assist in further understanding the mechanism and relevance of mouse lung tumor to human health assessment. This presentation will include a summary of the workshop. Disclaimer: The views expressed in this abstract are those of the authors and do not represent the policy of the US EPA.

P53

European Hot Spot of Air Pollution by PM2.5 and B[a]P: Ostrava, Czech Republic–New Knowledge, New Difficulties. Sram RJ[1], Rossner P[1], Rossnerova A[1], Libalova H[1], Milcova A[1], Schmuczerova J[1], Svecova V[1], Solansky I[1], Topinka J[1], Gmuender H[2]. [1]Institute of Experimental Medicine AS CR, Prague, Czech Republic, [2]Genedata AG, Basel, Switzerland.

Ostrava Region in the Northern Moravia (Silesia) is the most polluted region in the Czech Republic by particulate matter (PM10 and PM2.5) and carcinogenic polycyclic aromatic hydrocarbons (c-PAHs) as benzo[a]pyrene (B[a]P). Sources of this pollution are industry (steel production, coke oven), traffic, and local heating. In the most polluted district of Ostrava City Radvanice in the year 2012 was PM10 49.5±31.2 ug/m3, PM2.5 39.4±31.2 ug/m3, B[a]P was 10.8±13.7 ng/m3. Program Ostrava started in the year 2008 to study, what may be the impact of long-term exposure to c-PAHs to the health of population. The exposure to c-PAHs is very unique in all EU. One project was related to the asthma bronchiale in children, other to genetic damage in groups of volunteers. New project study the effect of exposure to air pollution to biomarkers in newborns as DNA adducts and gene expression profiles. Difficulties started, when we tried to inform about a possible health risk regional authorities: the incidence of asthma bronchiale in children from Ostrava-Radvanice seems to be the highest in all literature with the specific gene expression profiles; adult population may be affected by oxidative damage, which affect pathways for cardiovascular diseases, chronic exposure to high concentrations of B[a]P may induce an adaptive response. Exposure to B[a]P represents the most significant risk of air pollution in the Czech Republic. More than 60% of population is exposed to air pollution > 1 ng B[a]P/m3. Supported by the Grant Agency of the Czech Republic 301/13/458S.

P54

ZVI Mediated Removal of Cr(VI) and Phenol: A Sustainable Treatment Technology Coupling Chemical Redox System and Fentons AOP. Selvaraj A, Nambi IM. Indian Institute of Technolgy Madras, Chennai, TN, India.

Leachate coming out from urban waste dumpsite contains multiple organic compounds and heavy metals out of which Cr^{6+} were chosen as a representative heavy metal and phenol chosen as a representative organic compound. A new sustainable, effective, and long lasting treatment technology couples chemical oxidation reduction and advanced oxidation process system (AOPs) was used in combination with different forms of Zero Valent Iron (granular-reusing scrap, micron-synthesized from gZVI and nano sized-nanoferFe25S) to remove Cr^{6+} and Phenol. Coupled batch experiments were conducted as 1) sequential removal (Cr^{6+} first and Phenol first) representing planned mixed streams 2) concurrent removal (Cr^{6+} and Phenol together) representing premixed system. The quantification and chemical species form of iron and chromium in solid and ionic stage was also tracked throughout the reaction, helps in stoichiometric calculation for checking the mass balances and water quality assessments. Both Cr^{6+} reduction and Phenol oxidation were conducted with a new hypothesize that the iron can be made to recycle between the two reactions changing back and forth between Ferrous and Ferric forms which makes the treatment sustainable. The treatment process was successful and adequate iron remained in solution even up to three cycles of treatment. Low to neutral pH favored more iron and Cr^{3+} to remain in the dissolved phase which is advantageous for in-situ systems. Based on batch experiments, pilot scale in-situ treatment systems were developed and confirmed the speciation and precipitation and exhaustion of ZVIs related with pH of leachate offered effectyive ZVI usage consequently reduced the quantum of spent waste.

P55

Zidovudine (AZT) Induced Downregulation of hsa-mir-770-5p and Upregulation of STMN-1: A Novel Pathway to Aneuploidy. Crespo-Mejias Y[1], Rivera AV[1], Sanchez VC[2], Poirier MC[1], Olivero OA[1]. [1]Carcinogen-DNA Interactions Section, LCBG, CCR, National Cancer Institute, NIH, Bethesda, MD, United States, [2]In Vitro Pathogenesis Section, LCBG, CCR, National Cancer Institute, NIH, Bethesda, MD, United States.

Mechanisms underlying genomic instability induced by the antiretroviral drug Zidovudine (AZT) are not entirely understood. Our exploratory studies revealed that the microRNA hsa-miR-770-5p was downregulated in the mammary epithelial cell line MCF10A as a result of exposure to 100 or 200μM AZT for 24hr. We therefore chose to study the hsa-miR-770-5p target gene Stathmin1 (STMN1), because the concomitant upregulation of this gene would cause microtubule erosion and mitotic spindle destabilization, and we previously showed that 23% of normal human mammary epithelial cells exposed to 200μM AZT for 24 hr lacked the ability to polymerize microtubules. In these experiments we performed reverse transfections to introduce overexpression of hsa-miR-770-5p (defined as mimic) and inhibition of hsa-miR-770 (defined as inhibitor) in MCF10A cells. Cells, analyzed for STMN1 by RT-PCR, showed high levels of hsa-miR-770-5p in the mimics. Untreated mimic transfected cells were 70.1% positive for STMN1 by immunochistochemical (IHC) staining, and the untreated cells transfected with the inhibitor were 82.9% positive for STMN1 by IHC, confirming STMN1 expression increased when hsa-miR-770-5p levels were very low. AZT-exposed mimic transfected cells showed 54.9% of cells positive for STMN1, while AZT-exposed inhibitor transfected cells showed 49.5% of cells positive for STMN1. Western blot confirmed downregulation of STMN1 protein levels, by hsa-miR-770-5p overexpression in mimic transfected cells. In summary, down-regulation of hsa-miR-770-5p caused an increase in cells expressing STMN1, and AZT-induced genomic instability may occur through dysregulation of STMN1 and tubulin erosion.

P56

Translocation Breakpoints and G-quadruplex DNA. Williams J, Fleetwood S, Larson E. Illinois State University, Normal, IL, United States.

Translocations are caused by DNA breaks, but it is not clear why some loci are prone to this type of genetic instability. The oncogenic t(1;19) translocation occurs between the TCF3 and PBX1 genes. Here, we use the TCF3 and PBX1 loci as a model to better understand the role of G-quadruplex (G4 DNA) in genome instability. G4 DNA is a four stranded structure that folds from certain guanine repeats under physiological conditions, and it likely inhibits normal DNA metabolism. We asked if translocation break sites in TCF3 and PBX1 correspond with sequence motifs that support G4 DNA. We have found several repetitive guanine sequences that map to positions 3' and 5' of the TCF3 and PBX1 break point clusters. Synthetic oligonucleotides containing these sequences adopted G4 structures *in vitro*, as observed by native PAGE and Circular Dichoism. Structure formation was potassium dependent, consistent with G4 DNA. When the TCF3 and PBX1 break sequences were positioned to serve as templates for DNA synthesis, we observed potassium dependent stalling of Klenow polymerase on the guanine-rich, but not the cytosine-rich, strand *in vitro*. These results suggest that the t(1;19) translocation event may be influenced by the formation of G4 DNA structures. We favor a model where stalled replication or failed DNA repair at G4 DNA promotes DNA breaks, mutagenesis, and translocations. Beyond the t(1;19) translocation, our results may offer a molecular rationale for the genetic instability observed at other guanine-rich loci in the human genome.

P57

DNA Repair Defects and Centrosomal Deregulation Associated with Seckel Syndrome Microcephaly. Manchester DK[1], Olivero OA[2], Rivera A[2], Dauber A[3], Hwa V[4], Poirier MC[2], Spivak G[5]. [1]University of Colorado Denver School of Medicine, Aurora, CO, United States, [2]Carcinogen-DNA interactions Section, Laboratory of Cancer Biology and Genetics, National Cancer Institute, NIH, Bethesda, MD, United States, [3]Division of Endocrinology, Boston Children's Hospital, Boston, MA, United States, [4]Molecular and Medical Genetics and Pediatrics, Oregon Health and Science University, Portland, OR, United States, [5]Biology Department, Stanford University, Stanford, CA, United States.

Seckel syndrome is an autosomal recessive childhood disorder characterized by severe growth deficiency, microcephaly, distinctive facies, cognitive deficits, and increased risks for myelodysplasia. The disorder is genetically heterogeneous; seven complementation groups have been identified with mutations in proteins involved in damage response (ATR, ATRP), basal transcription (MAT1), and centrosomal function (CEP63, CEP152, CENPJ, PCNT, ninein). The *NIN* gene codes for ninein, a protein involved in anchoring centrosomes to microtubules. Dauber et al. (2012) reported *NIN* mutations in primordial dwarfs with Seckel syndrome. *NIN* has been assigned SCKL7 nomenclature. We follow a 20-year old female affected with microcephaly, cognitive delays, and photosensitivity. Although she has normal growth, her microcephaly and craniofacial features mirror Seckel syndrome though her clinical diagnosis is that of a Cockayne variant. However, all available CS and XP mutation analyses have been negative. DNA repair assays indicated that fibroblast survival curves were abnormal as was RNA synthesis recovery in UVC-exposed cells. Moreover, her cells were hypersensitive to treatment with menadione, an inducer of 8-oxo-Guanine in DNA. Whole exome sequencing has identified two heterozygous novel missense variants *in trans* in the *NIN* genes in our patient, consistent with a diagnosis of Seckel syndrome. We are currently comparing centrosomal stability in our patient with that of cells from the previous patients reported by Dauber et al. We have found that centrosomes are unstable in all of these patients indicating that they have pathogenic *NIN* mutations. The relationship(s) between these findings and UV sensitivity/DNA repair have yet to be determined.

P58

Novel Radiation Mitigators and Anticancer Drugs. Schiestl RH[1], Davoren M[1], Jung M[1], Rivina Y[2], Danilova N[1]. [1]University of California, Los Angeles, Los Angeles, CA, United States, [2]Stanford University, Palo Alto, CA, United States.

The possibility of a radiation disaster from a nuclear detonation or accident has existed for over 50 years, and the recent Fukushima accident was yet another reminder that there remains a dire need to develop novel therapies against radiation-induced toxicities. We report on the development of two novel radiation countermeasure therapies: Yel001 and Yel002. These small, biologically active, drug-like molecules were uncovered in the DEL high throughput assay reducing radiation-induced cyto- and geno-toxicity in yeast. Further, Yel compounds increases survival to 75% *in vivo* following an LD100/30 dose of ionizing radiation (IR) with the first therapeutic injection administered 24 hours post exposure followed by injections at 48,72,96, and 120 hours. Additionally, treatment with Yel001 and Yel002 compounds reduces radiation-induced leukemia from 90% to 50% and 40% respectively. Treatment with Yel002 following IR accelerates the recovery of the hematopoietic cells after sub-lethal exposures. In addition, treatment with Yel002 reduces EMS, MMS, UV, cigarette smoke extract as well as nitrogen mustard induced toxicity as well as genotoxicity showing a broad application spectrum. It also prolongs live of cells in a senescence assay. In addition *Atm* deficient mice live 16 weeks longer with weekly injection of Yel002 which is about 12 years in human life expectancy. In addition, Yel002 complements a zebrafish model of Diamond Blackfan Anemia. It works in yeast, CHO cells, different human cells, mice and zebrafish. Toxicity has not been observed in neither *in vitro* or *in vivo* administrations.

P59

High-Resolution SNP Genotyping Offers a Novel Genome-Wide Approach for Analysis of the Spatial Distribution of Mutations. Edge AK, Eitutis ST, Hill KA. Western University, London, ON, Canada.

Generally, mutations are rare, independent events. However, transient hypermutability results in elevated mutation frequency with *kataegis*, thundershowers or clusters of proximal mutations (>6 mutations with a maximum average inter-mutational spacing of 1Kb) repeated across the genome. Whole genome sequencing of cancer genomes identified *kataegis* using rainfall plots, where distance (bp) from the previous adjacent mutation is plotted against the genomic location of each mutation, colour-coded for point-mutation type. To date, *kataegis* has been identified only in some cancers of epithelial tissues, and it is hypothesized that transient hypermutability precedes carcinogenesis. Since *kataegis* occurs infrequently, SNP genotyping arrays provide a low cost alternative to whole genome sequencing for the necessary, large surveys using normal tissues. We used the Mouse Diversity Genotyping Array (over 500K SNPs from the C57Bl/6J reference genome) to detect admixture of C57Bl/6J and CBA/CaJ genetic backgrounds and track inheritance of linkage groups through mouse pedigrees. We determined that 82.7% of MDGA SNPs are proximal enough to detect *kataegis*. Rainfall plots were then used to display SNP differences detected for each of 15 C57Bl/6J healthy mice using tail, cerebellum and spleen samples. The spacing between the SNP differences is more than 1 million bp, with no observations of *kataegis*. Tissues predicted to have, although at low incidence, transient hypermutability and thus evidence of *kataegis* are epithelial tissues. We advocate the use of Rainfall plots of MDGA data as an efficient survey method for identifying shifts mutational landscapes resulting from genomic instability and environmental mutagen exposures.

P60

AP Endonuclease 1 Cooperates with Flap Endonuclease 1 to Remove a Trinucleotide Repeat Hairpin. Beaver JM, Xu M, Laverde E, Liu Y. Florida International University, Miami, FL, United States.

Oxidized DNA base lesions are induced by stress produced during cellular energy metabolism and from environmental toxicants and ionizing radiation. Base excision repair (BER) of a DNA base lesion within a trinucleotide repeat (TNR) tract can lead to instability of TNR length as a result of the formation of DNA secondary structures such as hairpins. We have found that BER of a base lesion in a TNR loop can remove the hairpin, attenuating or preventing TNR expansion. In this study, we provide the first evidence that AP endonuclease 1 (APE1) cooperates with flap endonuclease 1 (FEN1) to resolve a double-flap intermediate that forms during BER within a TNR hairpin loop. This promoted removal of the hairpin and prevention of TNR expansion. Further characterization of APE1 demonstrated that the 3' to 5' exonuclease activity of APE1 shortened the 3'-flap of the double-flap intermediate formed during BER within a large hairpin loop, resulting in a shortened flap that can be cleaved more efficiently by FEN1. Our study provides new insight for the role of APE1 in removing a TNR hairpin, thereby preventing TNR expansions during BER of DNA base lesions within TNR tracts.

P61

A 5', 8-deoxypurine Lesion Induces Trinucleotide Repeat Deletion via DNA Polymerase β. Xu M[1], Jiang Z[1], Terzidis M[2], Masi A[2], Chatgilialoglu C[2], Liu Y[1]. [1]Florida International University, Miami, FL, United States, [2]I.S.O.F., BioFreeRadicals Consiglio Nazionale delle Ricerche, Bologna, Italy.

5',8-cyclo-deoxypurines are common forms of oxidized DNA lesions resulting from endogenous and environmental oxidative stress such as ionizing radiation and can only be repaired by nucleotide excision repair with a low efficiency. This results in the accumulation of the lesions in the genomic DNA, thereby leading to stalling of replication DNA polymerases and poor lesion bypass by translesion DNA polymerases. Trinucleotide repeats (TNR) are tandem repeats of Gs and As and are therefore susceptible to formation of 5',8-cyclo-deoxypurines. In this study, we provided the first evidence that 5',8-cyclo-dA in a CAG repeat tract induced the formation of a small CAG loop, and this further promoted DNA polymerase β (pol β) skip-over of the loop structure, thereby exclusively resulting in a CTG repeat deletion during DNA lagging strand maturation and base excision repair. Moreover, we found that pol β bypass of the template 5',8-cyclo-dA resulted in repair deletion products of varying sizes, suggesting the formation of various sizes of CAG repeat loops that were bypassed by pol b resulting in a long flap. Subsequently, flap endonuclease I (FEN1) efficiently removed the flap leading to various sizes of repeat deletion. Our study suggests that long-term accumulation of 5',8-cyclo-deoxypurines in the human genome can result in TNR instability that ultimately causes human cancer and neurodegeneration. Our study provides new insight into the mechanisms of TNR instability induced by oxidative DNA damage and mediated by pol β during DNA replication and base excision repair.

P62

DNA Polymerase β Plays a Predominant Role in Bypassing a 5', 8-deoxypurine Lesion during DNA Replication and Base Excision Repair. Jiang Z[1], Xu M[1], Terzidis M[1], Masi A[2], Chatgilialoglu C[2], Liu Y[1]. [1]Florida International University, Miami, FL, United States, [2]I.S.O.F., BioFreeRadicals Consiglio Nazionale delle Ricerche, Bologna, Italy.

5',8-2'-deoxycyclopurines including 5',8-cyclo-dA and 5',8-cyclo-dG are induced by hydroxyl radicals resulting from oxidative stress such as ionizing radiation. Cyclodeoxypurine can be repaired by nucleotide excision repair inefficiently, thereby accumulating in the human genome. In this study, for the first time, we discovered that DNA polymerase b (pol β), efficiently bypassed a (*5'R*)-cdA or (*5's*)-cdA. We found that pol β knock-out MEF cell extracts failed to exhibit any DNA synthesis activity to bypass the lesions. Pol β wild-type and pol b overexpression cell extracts exhibited lesion bypass in DNA replication and base excision repair (BER) intermediates. This indicates that pol β can bypass a cyclo-dA during DNA replication and BER. Moreover, we found that 1 nM pol b inserted a correct nucleotide to bypass a cyclo-dA. However, 10 and 50 nM pol b inserted an incorrect nucleotide to bypass the lesion, indicating a mutagenic effect from high level of pol b. Our study provides the first evidence that pol β plays a predominant role in bypassing a 5',8-cyclodA during DNA replication and repair. Our results also provide new insight into a mutagenic effect by pol β bypassing of a 5',8-cyclo-dA that may underlie cancer genome instability and drug resistance.

P63

MSH2-MSH3 Promotes GAA Repeat Expansion by Stimulating DNA Polymerase β Activity during Base Excision Repair. Lai Y[1], Beaver JM[1], Chan NLS[2], Zhang Z[3], McMurray CT[2], Liu Y[1]. [1]Florida International University, Miami, FL, United States, [2]Lawrence Berkeley National Laboratory, Berkeley, CA, United States, [3]Sichuan University, Chengdu, China.

Expansion of GAA·TTC repeats within the first intron of the FXN gene is the cause of Friedreich's ataxia (FRDA), an autosomal recessive neurodegenerative disorder. However, no effective treatment for the disease has been developed yet because of lack of understanding mechanisms underlying GAA·TTC repeat expansion. Recent studies demonstrate a somatic instability of expanded GAA repeats in postmitotic tissues pointing to an important role of DNA repair in modulating somatic GAA repeat instability. It has been shown that the mismatch repair protein complex MSH2-MSH3 promotes GAA repeat expansion by binding and stabilizing non-B form secondary structures formed by GAA repeats. It remains unclear as to how MSH2-MSH3 may facilitate expansion of GAA·TTC repeats. In this study, we discovered that MSH2-MSH3 complex suppressed GAA repeat deletions during base excision repair (BER) in the context of $(GAA)_{20}$ repeats. In the absence of MSH2-MSH3, BER mainly resulted in a large deletion of 8 repeats along with only a limited size of expansions. This resulted from the formation of a large loop on the template stand and small bubbles on the damaged strand. Surprisingly, we found that MSH2-MSH3 directly stimulated the DNA synthesis by DNA polymerase β (pol β), and this facilitated pol β to go through the large loop structure formed on the template strand of GAA repeates, thereby suppressing repeat deletion and sustaining small repeat expansion. Our results demonstrate an interplay between mismatch repair and BER in modulating the instability of GAA repeats.

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Human MutS Homologue hMSH5 Promotes Homologous Recombination-Mediated Repair of Camptothecin-induced DNA Double-Strand Breaks. Xu Y, Wu X, Her C. Washington State University, Pullman, WA, United States.

The topoisomerase I inhibitor camptothecin (CPT) is a cytotoxic quinoline alkaloid anticancer agent, originally isolated from Camptotheca (Happy Tree in Traditional Chinese Medicine), that induces replication fork collapse and double-strand breaks (DSBs) amid DNA replication. The repair of CPT-induced DSBs is largely dependent on homologous recombination (HR). Despite the prominence of this agent in cancer therapy, the mechanisms underpinning the repair of CPT-induced DNA damage remain elusive. Here, we report that hMSH5 plays an important role in the processing of CPT-induced DNA lesions. Our study indicates that hMSH5 promotes cell survival in response to CPT treatment. The results of our clonogenic assays show that hMSH5 depletion sensitizes cells to CPT and cisplatin. In addition, hMSH5-deficient cells show elevated numbers of g-H2AX and RPA2 foci in response to CPT treatment with a concomitant reduction of hRad51 foci – indicative of impaired HR. These observations are consistent with the result obtained from HR reporter analysis, which demonstrates an HR-promoting role for hMSH5. Furthermore, hMSH5 deficiency leads to defective Chk1 and Chk2 activation, and consequently abnormal cell cycle progression, in cells treated with CPT. Since the Fanconi anemia (FA) pathway is required for the repair of both CPT-induced DNA strand breaks and DNA crosslinks, a functional interaction between hMSH5 and the FA pathway is presently under investigation with a particular emphasis on the avoidance of toxic DSB repair. Collectively, our data supports a role of hMSH5 in mediating DSB repair of collapsed replication forks and thereby the maintenance of genomic stability.

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Abstract Withdrawn.

P66

Molecular Signature and DNA Damage Pathways Altered in Male Largemouth Bass (*Micropterus salmoides*) By a Single Dose of Benzene and Trichloroethylene. Colli-Dula RC[1], Mehinto AC[2], Kroll KJ[1], Barber DS[1], Vulpe CD[3], Denslow ND[1]. [1]University of Florida, Gainesville, FL, United States, [2]Southern California Coastal Water Research Project, Costa Mesa, CA, United States, [3]University of California, Berkley, Berkley, CA, United States.

Benzene (BZ) and trichloroethylene (TCE) can be found in aquatic systems contaminated by industrial discharges. Largemouth bass (LMB) are an important fish model used in ecotoxicology. The modes of action (MoA) of TCE and BZ are established in mammals, but unclear in fish. We applied transcriptional profiling to evaluate the response of LMB to BZ and TCE to clarify the MoA and potential ecological impact. Oligo microarrays were used to identify key genes and pathways in the liver and gonad of LMB exposed to an acute dose (10 mg/kg b/w) of BZ and TCE. We identified similarities in responses between both treatments in the liver but not in the gonad. In both BZ and TCE treated fish, genes related to detoxification, apoptosis, cell checkpoint pathways, and tumorigenesis/cancer were significantly altered in the liver. Gene ontology (GO) analysis revealed potential effects on mitosis, DNA replication, and response to DNA damage from stimulus in the liver. Gene Set Enrichment Analysis (GSEA) of the liver suggested that DNA Replication and Single-Strand Base Excision DNA Repair were affected following exposure. In addition, Sub-Network Enrichment Analysis (SNEA) suggested that cell processes such as mitotic spindle checkpoint, and DNA replication checkpoint were significantly affected by both treatments. In contrast, affected gonadal GO categories were specific to each treatment and GSEA of gonadal tissue determined that the glutathione metabolism pathway was affected in both treatments. This study suggests that both BZ and TCE induce DNA damage in LMB, consistent with effects and mechanisms found in mammalian models.

P67

A 5', 8-deoxypurine Lesion Induces Genome Instability via DNA Polymerase β during DNA Replication and Base Excision Repair. Liu Y[1], Xu M[1], Jiang Z[1], Terzidis M[2], Masi A[2], Chatgilialoglu C[2]. [1]Department of Chemistry and Biochemistry, Florida International University, Miami, FL, United States, [2]I.S.O.F., BioFreeRadicals Consiglio Nazionale delle Ricerche Via P. Gobetti, Bologna, Italy.

5',8-cyclo-2'-deoxynucleosides including 5',8-cyclo-dA and 5',8-cyclo-dG are common forms of oxidized DNA lesions resulting from endogenous and environmental oxidative stress such as ionizing radiation. 5', 8-cyclo-deoxynucleosides can be repaired by nucleotide excision repair with a low efficiency. This results in accumulation of the lesions in the genomic DNA, thereby leading to stall of replication DNA polymerases and poor lesion bypass by translesion DNA polymerases. In this study, we provided the first evidence that DNA polymerase β (pol β) plays a predominant role in bypassing a 5',8-cyclo-dA. We found that mouse embryonic fibroblasts (MEF) that express pol β exhibited an efficient and moderate bypass of a 5'R-cyclo-dA and 5's-cyclo-dA, respectively. In contrast, pol β knock-out MEFs exhibited poor lesion bypass activity. This indicates that the polymerase is involved in mediating 5',8-cyclopurine lesion bypass in cells. We showed that a moderate level of pol β inserted a correct nucleotide to bypass a 5's- and 5'R-cyclo-dA. However, a high level of pol β misincorporated nucleotides to bypass the cyclopurine lesions resulting in mutagenesis. Further characterization of the roles of cyclo-dAs on CTG repeat stability showed that the lesions preferentially caused CTG repeat deletion by inducing the formation of a small CAG repeat loop at the template strand that further led to the formation of a downstream flap. This promoted pol β skip-over of the loop and FEN1 flap cleavage leading to repeat deletion. Our study provides a new insight to genome instability induced by cyclo-deoxypurines during DNA replication and repair.

P68

Correlation between *CYP1A1* RNA Transcript, Protein Level, Enzyme Activity, and DNA Adducts in Primary Normal Human Mammary Epithelial Cells Exposed to Benzo[a]pyrene. Divi RL[1], Lindeman TE[1], Shockley ME[1], Keshava C[2], Weston A[3], Poirier MC[1]. [1]National Cancer Institute, Bethesda, MD, United States, [2]National Center for Environmental Assessment, EPA, Research Triangle Park, NC, United States, [3]Division of Respiratory Disease Studies, NIOSH, CDC, Morgantown, WV, United States.

Benzo(*a*)pyrene (BP) undergoes metabolic activation and forms DNA adducts. The goal of this study is to identify the key players that contribute to BP-DNA adduct formation in mammary epithelial cells. We quantified RNA copies/ng cDNA (RNA cpn) of *Cytochrome P450 1A1 (CYP1A1) and CYP1B1,* genes which code for metabolic enzymes that form r7, t8, t9-trihydroxy-c-10-(N^2-deoxyguanosyl)-7,8,9,10-tetrahydro-benzo[*a*]-pyrene (BPdG), the major BP-DNA adduct, and *NAD(P)H:Quinone Oxidoreductase 1 (NQO1),* which codes for NQO1 that converts BP quinones to less toxic hydroquinones. Primary normal human mammary epithelial cell (NHMEC) strains from 16 healthy women and MCF-7 breast cancer cells were used for comparison. We found 56-836 and 251-13234 *CYP1A1*, 336-5587 and 4133-57077 *CYP1B1*, and 5943-40112 and 4456-55887 *NQO1* RNA cpn in unexposed and BP exposed (4 µM, 12h) NHMECs, respectively. NHMECs had 7.47 (median; range: 0.85-15.8) BPdG adducts/10^8 nucleotides while MCF-7 cells had 790 adducts. In the NHMECs, a linear association (p=0.0015) was observed between BPdG adducts and BP-induced *CYP1A1*, and no correlation with other genes examined. Western blots of 4 NHMEC strains, chosen for different levels of BPdG adducts, showed a linear correlation (p=0.013) between BPdG and CYP1A1, but none between BPdG and CYP1B1 or NQO1. Ethoxyresorufin-O-deethylase (EROD) activity, which measures CYP1A1/1B1 together, correlated (p=0.038) with BPdG in NHMECs, and was highly induced by BP in MCF-7 cells. Overall, the data suggest that CYP1A1 is critical for BPdG adduct formation in NHMECs. The 10-fold higher adduct level found in BP-exposed MCF-7 cells, compared to NHMECs, appears likely due to high EROD activity.

P69

Use of Directed Evolution to Study Substrate Discrimination by ALKBH2. Lepore AL, Troll C, Alexander DL, Camps M. University of California, Santa Cruz, Santa Cruz, CA, United States.

The human DNA repair enzyme ALKBH2 is a direct repair enzyme that acts to remove cytotoxic methyl damage and mutagenic etheno adducts from DNA. While previous research has uncovered many important structural features of ALKBH2, it is still unknown how the enzyme discriminates between these two types of DNA damage given that once they are flipped into the active site of the enzyme, these two lesions occupy nearly identical positions in the active site. In order to identify key residues involved in the discrimination of methyl and etheno lesions, we are screening a collection of ALKBH2 mutants previously selected for increased protection to exposure to the methylating agent MNNG for differential methyl versus etheno repair. As a screening method, we use lysogenic infection of a phage treated with the SN2 methylating agent methyl methane sulfonate (methylation repair) or chloroacetaldehyde (etheno repair). We identified mutants in this library that selectively reduce methyl repair while leaving etheno repair, and plan to confirm these results genetically by looking at differences in mutation expression and biochemically by mass spectrometry. The identification of mechanisms of substrate discrimination for ALKBH2 should facilitate the production of adjuvant ALKBH2 inhibitors specific for methyl repair. These inhibitors would enhance the therapeutic effect of methylating agents while minimizing the potential carcinogenic effects of etheno-induced mutagenesis.

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Low-Dose H_2O_2 Induced Clustered DNA Lesions and Mutagenesis: Contribution of Error-Prone NHEJ Repair Pathway. Sharma V, Collins LB, Swenberg JA, Nakamura J. University of North Carolina, Chapel Hill, NC, United States.

Although the induction of oxidatively induced clustered DNA lesions (OCDLs) has been believed as a finger print of radiation-induced DNA damage, few studies have also associated elevated levels of OCDLs with chronic inflammation and human malignancies. There is a knowledge gap regarding formation of OCDLs/DSBs as a result of low levels of endogenous/exogenous oxidative stress (OS) and their role in mutagenesis. Therefore, in the present study, we sought to understand the generation of OCDLs and OS induced mutagenesis caused by low levels of H_2O_2 and identify DNA repair pathways that may affect OS induced susceptibility to mutagenesis. Low concentrations of H_2O_2 actually found in cells during inflammatory processes were taken. Interestingly, DNA Damage Response analyses in DT40 cells, using reverse genetic approach, revealed hypersensitivity of Rad54, Rad51c, XRCC2, Ku70 and Lig IV deficient cells to H_2O_2, indicating the potential role of DSBs in H_2O_2 toxicity. High levels of 8-oxo-dG lesions and OCDLs measured with a modified PFGE version were also found in H_2O_2 exposed cells. The induction of OCDLs and DSBs directed us to investigate the role of error-prone NHEJ in mutagenesis. Ku70, DNA PKcs and Lig IV (NHEJ proteins) deficient cells revealed a drastic decrease in mutation frequency despite the presence of equivalent levels of 8-oxo-dG as in the wild-type DT40 cells. Our results indicate that OS, even at low levels, can cause clustered DNA damage that leads to DSBs with complex DNA ends and repairing such complex DSBs with NHEJ increases the likelihood that mutations will result.

P71

BPA Modulates Repair of Oxidative DNA Damage by Base Excision Repair Pathway. Gassman NR, Stefanick DF, Horton JK, Wilson SH. Laboratory of Structural Biology, National Institute of Environmental Health Sciences, National Institute of Health, Research Triangle Park, NC, United States.

Bisphenol A (BPA) has become a target of intense public scrutiny since concerns about its association with human diseases such as obesity, diabetes, reproductive disorders, and cancer have emerged. Several recent studies have linked genotoxicity of BPA with the generation of oxidative DNA damage. Reactive oxygen species (ROS) that damage DNA are generated by metabolism of BPA and can generate DNA strand breaks and damaged DNA bases. Base excision repair (BER) is responsible for removing oxidative base lesions, such as 8-oxo-2'-deoxyguanosine (8-oxoG), and repairing single strand breaks (SSBs), yet the relationship between BPA and BER has yet to be examined. Further, the ubiquitous nature of BPA causes continual exposure of the human genome concurrent with the normal endogenous and exogenous insults to the genome, and this co-exposure may impact DNA damage response and repair. To determine the effect of BPA exposure on repair of oxidative DNA damage, DNA repair proficient and deficient cells lines were co-exposed with BPA and the oxidizing agent, potassium bromate. Repair-deficient cell lines were found to be more sensitive to the generation of oxidative damage; however, in the presence of BPA an enhanced cell survival was observed coupled with increases in the 8-oxoG content of the DNA. This protective effect and increased DNA lesion load resembles a DNA glycosylase-deficient cell phenotype, suggesting that initiation of BER is suppressed by BPA. The role of BPA in suppression of DNA repair and reduction of cell death will be discussed.

P72

Loss of DNA Polymerase Beta Induces Premature Senescence: A Role for BER in Accelerated Aging in Down Syndrome. Ahmed AA, Park-York MJ, Cabelof DC. Wayne State University, Detroit, MI, United States.

Down syndrome (DS) is a condition of intellectual disability characterized by accelerated aging. The mechanism(s) of accelerated aging remain mechanistically unknown, in spite of evidence that DNA polymerase beta (polB) is reduced in DS. We hypothesize that loss of polB drives senescence through a mechanism involving miR-155, a chromosome 21 localized microRNA. We evaluated hydroxyurea induced senescence in polB null versus polB wildtype primary cells, and find that loss of polB significantly increases the percentage of senescent cells (3-fold increase, $p<0.01$). We also find senescent cells in polB null cells in response to methane methylsulfonate (MMS) while WT cells do not exhibit any senescence. Thus we establish that loss of polB induces senescence. Individuals with DS display reduced levels of polB. Accordingly, we evaluated senescence in primary fibroblasts from DS donors. DS and nonDS primary fibroblasts were exposed to 100μM H_2O_2 for 5 days, resulting in a two-fold increase in senescence in the DS fibroblasts ($p<0.01$). This was accompanied by an increase in p16 expression (1.8 fold increase, $p<0.01$). We suggest that miR-155 inhibits polB inducibility. To test this, we ectopically overexpressed miR-155 at levels consistent with a gene dosage effect of trisomy 21. We find that miR-155 overexpression downregulates polB expression (~50%, $p<0.001$). Next, we transfected miR-155 overexpressing cells with polB promoter and find a significant reduction in polB promoter activity in response to MMS (40% reduction, $p<0.05$) and H_2O_2 (50% reduction, $p<0.05$), suggesting that miR155 may play a key role in inhibition of polB and promotion of senescence.

P73

Regulation of Base Excision Repair in Genome Maintenance. Limpose K, Werner E, Corbett AH, Doetsch PW. Emory University, Atlanta, GA, United States.

Dysregulation of DNA repair and genome maintenance pathways contribute to cancer by increasing levels of DNA damage. Ultimately, unrepaired DNA damage results in the accrual of mutations and genomic instability. A common cause of DNA damage is reactive oxygen species (ROS) that are generated from normal cellular metabolism, inflammation, or through exogenous sources such as radiation. Importantly, tumor cells experience an increased ROS burden from these sources, potentially facilitating more mutations. Base excision repair (BER) is the main pathway for repairing ROS-induced DNA damage, and is initiated by the *N*- glycosylase proteins. How BER dysregulation contributes to cancer development is largely unknown. To investigate this question, the NTHL1 glycosylase is being employed as a model to address the mechanisms of BER regulation and the role of BER in preventing cancer. Possible modes for NTHL1 regulation are compartment-specific localization and/or posttranslational modification(s) in response to ROS-induced DNA damage. BER dysregulation is being tested through biochemical and cellular assays. This study provides insight into BER mechanisms that contribute to genome integrity, and how BER dysregulation contributes to tumorigenesis.

P74

A Human Short ORF-Encoded Peptide That Interacts with Ku and Stimulates Nonhomologous End Joining. Hanakahi LA[1], Slavoff SA[2], Heo J[1], Budnik BA[2], Saghatelian A[2]. [1]University of Illinois, Rockford, IL, United States, [2]Harvard University, Cambridge, MA, United States.

Peptidomic discovery of numerous Short open reading frame-Encoded Polypeptides (SEPs) in human cells suggests that one, or more, of these small proteins may play important roles in DNA repair. We found that the 69-amino acid (7.5 kDa) SEP MRI-2 physically interacts with Ku and stimulates non-homologous end joining (NHEJ) *in vitro*. Nuclear localization of MRI-2 was influenced by Ku expression. Treatment with etoposide resulted in increased stability and nuclear localization of MRI-2. Our data suggest that this small protein may play an important role in double-strand beak repair by NHEJ and highlights the potential biological relevance of SEPs in the DNA damage response and in DNA repair.

P75

The Effect of Changing Deoxyribonucleotide Concentrations on DNA Polymerase η Fidelity. 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 (8-oxoG) during DNA replication. When copying DNA, error-prone pol η frequently misincorporates incorrect nucleotides contributing to mutagenesis and genomic instability. As relative dNTP concentrations can affect the rate of nucleotide misincorporation, we have investigated the role of changing dNTP concentrations on pol η's error rate and hypothesized that nucleotide concentrations that attempt to approximate mammalian physiological ratios would alter rates of single base substitutions when copying both undamaged and damaged DNA. To study the effect of these mutations, we expressed the catalytic core of wild type human pol η in *E. coli*. 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 were used in *in vitro* assays to evaluate DNA synthesis opposite templates with and without 8-oxoG and CPD lesions. Preliminary experiments show that the efficiency of primer elongation is limited when dNTP concentrations approximate physiological concentrations and that these concentrations modify the error rates and mutation spectrum observed. Because dNTP concentrations vary throughout the cell cycle, we propose that these results suggest that the fidelity of pol η is dynamic. Furthermore, we believe that the findings are important to other DNA metabolic pathways in which pol η has been implicated such as noncanonical mismatch repair that may be occurring outside S-phase when dNTP concentrations are low.

P76

Cdc25A Acetylation As a Response to Environmental Challenge. Lozada EM, Yin M, Redilla N, Stambrook P. University of Cincinnati, Cincinnati, OH, United States.

Altered control of cell cycle regulation is an important cellular response to exposure to environmental agents, particularly genotoxicants. A critical participant in this regulatory process is the dual specificity phosphatase Cdc25A, which is a member of the Cdc25 family of phosphatases. These phosphatases play a key role in cell-cycle progression by dephosphorylating and activating cyclin-dependent kinases. In response to treatment with environmental genotoxins, Cdc25A is subject to posttranslational modifications which contribute to its proteasome-mediated degradation. For many proteins, posttranslational modification is often required for their normal biological function. The most thoroughly studied of these posttranslational modifications is phosphorylation, which has been reported for Cdc25A. Here, we provide evidence for the first time that Cdc25A can be acetylated *in vivo* and directly interacts with the transacetylase ARD1. We further show that arsenic, an environmental genotoxicant, increases Cdc25A acetylation. Taken together, it is likely that Cdc25A is regulated by acetylation, which in turn may be related to a specific cellular response to DNA damage. We intend to further clarify the relationship between Cdc25A acetylation and its function in cell cycle regulation and genomic integrity. Together, our studies advance our understanding of the dynamics of Cdc25A modification in response to environmental challenge and DNA damage and ask whether, and to what extent, acetylation of Cdc25A contributes to genomic integrity surveillance. Since Cdc25A is frequently overexpressed in multiple types of cancer, our findings may also point to mechanisms that underlie carcinogenesis.

P77

Exploring the Mechanism of SN1 Methylating Agent Protection by ALKBH2. Lilly JP, Alexander DL, Camps M. University of California, Santa Cruz, Santa Cruz, CA, United States.

ALKBH2 is DNA repair gene that removes two cytotoxic lesions from DNA: 1methyladenine (1MeA) and 3methylcytosine (3MeC). This enzyme is an iron and 2-oxoglutarate- dependent dioxygenase, which removes the cytotoxic methyl groups mentioned above in an error-free, single-step reaction. 1MeA and 3MeC are lesions that are made by weak alkylating agents, such as methyl donors generated by endogenous cellular processes. Methylating agents used for cancer treatment, such as temozolomide, produce different cytotoxic lesions, notably 3methyl-adenine (3MeA) and O^6-methylguanine (O^6MeG). Therefore, the therapeutic effect of these agents was initially not thought to be affected by ALKBH2. Against this expectation, several groups have recently reported that ALKBH2 expression protects glioma cell lines from temozolomide toxicity and the mechanism of protection is still unknown. Here we explore ALKBH2 protection using MNNG as a direct methylating agent that mimics temozolomide. Specifically, we address the following two questions: 1) Does ALKBH2-mediated resistance in K562 cells require ALKBH2 catalytic activity or is it indirect? 2) Can ALKBH2-mediated protection be enhanced by directed evolution? We will show the results of MNNG-selection of random ALKBH2 libraries in an AlkB-deficient strain of *E. coli*, which produced mutants that confer moderate protection in *E. coli* from 3MeA toxicity.

P78

An *In Vitro* Study on the Carrier-Like Function of Human Erythrocytes for Transferring Oxidative Stress from Exogenous Formaldehyde. Mei Y[1], Wei C[1], Zhang L[2], Yang X[1]. [1]Central China Normal University, Wuhan, Hubei, China, [2]University of California, Berkeley, Berkeley, CA, United States.

Introduction: Formaldehyde (FA) has been reportedly associated with leukemia. Previous studies showed that FA levels were not changed in the blood of exposed humans. The current study was, thus, designed to investigate whether or not blood has a buffering effect on exogenous FA, if so, what roles erythrocytes may play. Methods: Human healthy blood was obtained as experimental material. Various concentrations of FA (0, 0.03, 0.05, 0.125, 0.25, 0.5, 1, 2, 4, 8 and 16 mg/L) were prepared with 4% FA solution and dispersed in double-distilled water, saline, plasma and whole blood. After water bathing for 1h at 37°C, FA concentration in all prepared solutions was measured using 4-amino-3-hydrazino -5mercapto-1,2,4-triazole method. The carbonylation value of erythrocyte membrane protein in the blood was also determined with a 2,4-dinitrophenylhydrazine method, while malondialdehyde (MDA) content was measured to indicate the lipid peroxidation of erythrocyte membrane. Results: Doubled-distilled water and saline did not show any effect on exogenous FA, however, plasma has a buffering function when exogenous FA concentrations were prepared <2.0 mg/L and whole blood has the function even at the highest dose of 16 mg/L FA. Exogenous FA (≥0.25 mg/L) led to a significant increase in carbonylation value of erythrocyte membrane protein and MDA levels. Conclusions: Based on our empirical data, whole blood has a buffering effect on exogenous FA that at higher concentrations (2.0~16 mg/L) can cause the carbonylation in erythrocytes. Erythrocytes may play a major role in performing a possible carrier-like function for transferring oxidative stress of exogenous FA molecule.

P79

Immuno-Modulatory Role of N-Acetylcysteine in Cadmium Treated Human Lung Cells. Odewumi CO[1], Latinwo LM[1], Ruden M[1], Badisa VL[1], Fils-Aime S[1], Abdullah A[2]. [1]Florida A&M University, Tallahassee, FL, United States, [2]Bethune-Cookman University, Daytona Beach, FL, United States.

Cadmium Chloride ($CdCl_2$) exposure has been linked to lung cancer which is the second leading cause of cancer related deaths in the United States and developed countries. The anti-oxidant and anti-inflammatory properties of N-acetyl-cysteine (NAC) were evaluated in A549 human lung cells. Experimental cells were divided into four groups: control, Cd-only, NAC-only, and $CdCl_2$ plus NAC. The results showed that cells viability was significantly decreased to 44.5% in the cells treated with $CdCl_2$ alone in comparison to the untreated control cells (100%), NAC effectively ameliorated the $CdCl_2$ adverse effect of cell viability. The cytokines expression measured by Ray Biotech arrays and confirmed by the ELISA showed differential expression patterns. In the $CdCl_2$ alone treated cells, 19 cytokines were up regulated (expression 30% above control) and 8 cytokines were down regulated (expression 30% below control). The cytokines that were up regulated in the Cd alone treated cells were BDNF, eotaxin-2, FGF-6, FGF-7, Fit-3 ligand, GCP-2, GDNF, GM-CSF, IFN-γ, IGFBP-1, IGF-1, IL-1α, IL-3, IL-4, IL-5, IL-10, IL-13, IL-15, and IL-16. In the NAC co-treated cells, all the above cytokines expressions were reduced in comparison to $CdCl_2$ alone. The cytokines that were down regulated in the $CdCl_2$ alone cells were CNTF, EGF, eotaxin, IGFBP-2, NT-3, PARC, PDGFBB, and TGF-β3. Conversely, majority of the of the above cytokines except for the TGF-β3 that were down regulated in $CdCl_2$ alone were up regulated in the presence of NAC. These results showed the immune-modulatory role of NAC on cytokines expression in cadmium treated human lung cells.

P80

Epigenetic Transgenerational Inheritance of Male Fertility in the Rat Induced by the Endocrine Disruptor p,p'-DDE. Song Y, Wu N, Wang S. Zhejiang Academy of Medical Sciences, Hangzhou, China.

p,p'-dichlorodiphenoxydichloroethylene (p,p'-DDE), the major metabolite of dichlorodiphenoxytrichloroethane (DDT), is a known endocrine disruptor chemical and male reproductive toxicant. It remains unclear the transgenerational effects on male fertility and the possible epigenetic mechanisms. Only pregnant rat (F0) was administered with p,p'-DDE (daily gavage of 100 mg/kg body weight) or corn oil at the time of embryo sex determination (from gestation day 8 to 15). Male rats of F1 generation were mated with female to produce F2 progeny. To determine whether the transgenerational phenotype was transmitted through the male germ line, F3 progeny was generated by intercrossing the control and treated male and female of F2 generation and divided as following groups: 1) C♂-C♀, 2) DDE♂-DDE♀, 3) DDE♂-C♀, and 4) C♂-DDE♀. Male fertility impairment induced by p,p'-DDE could be observed in F1 generation and transferred to F3 generation through the male germ line. Up- and down-regulated *H19* and *Igf2*, caused by hypomethylation status of the differentially methylated region 2 (DMR2) of *Igf2*, may be one of the mechanisms underlying the transgenerational impaired fertility. Environmentally induced epigenetic transgenerational inheritance of male fertility appears to be a factor in disease etiology that needs to be considered and elucidated.

P81

Epigenetic Effects of Cadmium. Nath RG, Sonawane BR, Vulimiri SV, Lin Y-S. National Center for Environmental Assessment, Office of Research and Development, US EPA, Washington, DC, United States.

Cadmium (Cd) is a toxic metal and its exposure is known to be associated with various types of cancers and cardiovascular diseases. However, the underlying molecular mechanism(s) are unknown. Suggested mechanisms for the carcinogenic effect of Cd include aberrant gene expression, inhibition of DNA damage repair, induction of oxidative damage, inhibition of apoptosis, alteration of DNA methylation and aberrant microRNA expression. We conducted a review of literature to understand the epigenetic markers of Cd exposure from *in vitro* and *in vivo* experiments including human studies. *In vitro* studies in human immortalized myelogenous leukemia (K562) cells showed that Cd treatment led to increase in reactive oxygen species (ROS), DNA hypomethylation and cell proliferation. Based on results from studies with the antioxidant N-acetylcysteine and methionine, it was concluded that DNA hypomethylation rather than increase in ROS led to Cd-induced cell proliferation. In cultured rat liver cells (TRL 1215), short-term exposure to Cd inhibited DNA methyltransferase resulting in DNA hypomethylation, however, prolonged exposure induced hypermethylation. Chronic exposure to low dose Cd in rats and mice is associated with global hypermethylation and decreased hepatic apoptosis which could eventually lead to preneoplastic lesions. Occupational exposure to Cd-rich particulate matter in foundry workers resulted in significant changes in peripheral blood leukocyte microRNA expression (miR-222 and miR-21). Further studies are needed to delineate the epigenetic mechanisms involved in Cd-induced carcinogenesis. Disclaimer: The views expressed are those of the authors and do not necessarily represent the views and/or policies of the US EPA.

P82

Epigenetic Effects of Di(2-ethylhexyl) Phthalate. Sonawane BR, Nath RG, Makris SL. National Center for Environmental Assessment, Office of Research and Development, US EPA, Washington, DC, United States.

Epidemiological and laboratory investigations suggest that in addition to genetic changes, environmental pollutants can affect human health through altering epigenetic mechanisms including DNA methylation, histone modification and microRNA expression. There is evidence in animals and humans of an association of DEHP exposure and developmental and reproductive toxicity. Multiple lines of evidence from *in vitro* and *in vivo* models have established that epigenetic changes occur upon *in utero* exposures to several phthalates including di(2-ethylhexyl) phthalate (DEHP). We evaluated the published evidence for epigenetic changes by exposure to DEHP and its major metabolite mono(2-ethylhexyl)phthalate, formed by conversion of DEHP by intestinal lipases. Maternal exposure to DEHP has been shown to increase DNA methylation and expression levels of DNA methyltransferases in mouse testis. Further, some epigenetic effects of phthalates in female rats were found to be transgenerational. Exposure to a plastic mixture containing bisphenol A, dibutyl phthalate and DEHP during a period of embryonic sex determination in rats was shown to promote early onset of female puberty transgenerationally by decreasing the pool size of ovarian primordial follicles. These observations suggest that a mixture of plastic-derived compounds, can promote epigenetic transgenerational inheritance of diseases in adults. It is important to note that the mechanisms by which phthalates such as DEHP modulate changes in epigenetic markers are yet to be fully elucidated in experimental animals and humans. Disclaimer: The views expressed are those of the authors and do not necessarily represent the views and/or policies of the US EPA.

P83

Murine Perinatal Pb Exposure: Effects on Physiology and Epigenetic Drift over the Life Course. Faulk C, Barks A, Liu K, Anderson O, Goodrich J, Zhang Z, Sànchez B, Peterson K, Dolinoy D. University of Michigan, Ann Arbor, MI, United States.

Developmental lead (Pb) exposure is associated with lower body weight in human infants and late onset obesity in mice. Similarly, epigenetic patterning in imprinted genes is altered in adult humans coincident with undernutrition during early development. Here we measured physiological changes and DNA methylation shifts in association with perinatal Pb exposure in mice over the life course. Mice were exposed to 0, 2.1, 16, or 32 ppm Pb-acetate water two weeks prior to mating through lactation. Offspring were phenotyped at 3, 6, and 9 months of age for energy expenditure, spontaneous activity, food intake, body weight, and glucose tolerance. Tail DNA methylation was measured in 4 genes at weaning and again at 10 months. Both sexes exhibited increased energy expenditure compared to controls ($p<0.0001$). In females, horizontal activity differed significantly from controls ($p=0.02$). Overall, food intake increased in exposed females and males ($p<0.0008$ and $p<0.0001$) with significant linear trends at 9 months in females ($p=0.01$) and 6 months in males ($p<0.01$). Body weight was significantly increased in males at medium and high exposures ($p=0.001$ and $p=0.006$). Insulin response was significantly increased in males at medium exposure ($p<0.05$). DNA methylation levels in controls increased over time at imprinted *Igf2* and *Igf2r* loci (both $p=0.0001$), but not at imprinted *H19* locus or $Cabp^{IAP}$ metastable epiallele. Pb exposure was associated with accelerated DNA hypermethylation in $Cabp^{IAP}$ ($p=0.0209$) and moderated hypermethylation in *Igf2r* ($p=0.0447$). Our results indicate that developmental Pb exposure results in persistent changes in both the physiology and epigenetic marks in mice.

P84

Establishment and Use of a Cell Line for High-Throughput Screening of DNA Demethylating Agents. Toshikazu U, Okochi-Takada E. National Cancer Center Research Institute, Tokyo, Japan.

Aberrant DNA methylation is deeply involved in human cancers. Nevertheless, only a limited number of environmental agents capable of inducing aberrant DNA methylation have been identified. We previously developed a system to detect DNA demethylating agents [Okochi-Takada, *Mutat Res*, 568:187, 2004], but the system was suitable for high-throughput screening (HTS). Here, we aimed to establish a cell-based HTS system that detects DNA demethylating agents. First, we identified a gene whose promoter CpG island (pCGI) was heavily methylated, and that was re-expressed sensitively in response to a DNA demethylating agent, 5-aza-2'-deoxycitidine (5-aza-dC). Then, EGFP-IRES-LUC marker genes connected downstream of the pCGI was methylated and transfected into HCT116 cells. Three clones (HML58, 60, and 61) had an expected genomic structure, and showed demethylation and robust expression of the marker genes in response to low doses of 5-aza-dC. Although each of the three clones was genetically homogeneous, it was epigenetically heterogeneous as evidenced by heterogeneous GFP expression after 5-aza-dC treatment. We screened 300 subclones from the three clones, and isolated 14 subclones that showed uniform response to 5-aza-dC. Especially, a subclone (HML58-3) responded to as low as 0.025 μM of 5-aza-dC with high signal/noise ratio. The subclone was used to screen 19,840 small molecules, and we obtained four hit compounds, two of which were known DNA demethylating agents, 5-aza-dC and 5-aza-C (azacitidine). In conclusion, we established a cell-based HTS system of DNA demethylating agents.

P85

Effect of High and Low LET Radiation Exposures on DNA Methylation. Yu L, Baulch JE. Department of Radiation Oncology, University of California, Irvine, Irvine, CA, United States.

Epigenetic changes are mitotically and meiotically heritable stable, alterations in gene expression that include DNA methylation, histone modification and RNA-associated gene silencing. In mammals, 5-methylcytosine in DNA occurs at CpG dinucliotides and is normally associated with an inactive chromatin state and repressed gene activity. Recently it has also been shown that other cytosine modifications play a key role in regulating gene expression and cellular phenotype. These other modifications include 5-hydroxymethylcytosine, which has been found primarily in the brain and CNS. While DNA methylation is important for normal development, cell proliferation and genome maintenance, alterations in DNA methylation have emerged as one of the most consistent molecular alterations in multiple cancers. It has also been acknowledged that radiation exposure induces changes in DNA methylation. We have used *in vitro* irradiated cultured cells and *in vivo* irradiated mice to describe the effect of varied linear energy transfer (LET) radiations on tissue-specific DNA methylation profiles in the radiation response at delayed times after exposure. Differences in methylation profiles between cell lines or tissues, or over time following exposure provides mechanistic information regarding the radiation response and allows for the development of functional biomarkers for exposure and for carcinogenesis.

P86

Arsenic Epigenetically Regulates SLBP Which Leads to Aberrant Polyadenylation of H3.1 mRNA That Remains Present Outside of S Phase. Brocato JA, Chervona Y, Jin C, Costa M. New York University School of Medicine, New York, NY, United States.

Expression of canonical histone genes rapidly increases at the G1/S phase transition and maintains high levels until the end of S phase. Unlike transcripts of other genes, canonical histone mRNAs do not contain a poly(A) tail but instead display a stem loop structure at their 3' end. This stem loop structure is the binding site of the stem loop binding protein (SLBP), which increases the stability of the mRNA and facilitates its translation. Here we show that arsenic exposure depletes cellular levels of SLBP by inducing its proteasomal degradation and decreasing SLBP gene expression via epigenetic mechanisms at its promoter. Cells respond to the absence of SLBP by inducing aberrant polyadenylation of H3.1 mRNA, which increases the half-life and facilitates translation of the mRNA, two factors that provide for the observed increase of H3.1 protein levels. The poly(A) H3.1 mRNA is not susceptible to normal degradation that occurs at the end of S phase and we demonstrate its continued presence into M phase. This is the first study to describe aberrant polyadenylation of H3.1 mRNA to compensate for a loss of SLBP induced by arsenic exposure. The presence of H3.1 outside of S phase may interfere with nucleosome remodeling involving H3.3 and consequently interfere with gene expression, cell cycle control, and genomic instability.

P87

Multiple Types of DNA Damage in Germinal Center-Derived Human B-Cell Lymphomas That Express AID. Shalhout SZ[1], Sosin A[2], Martin A[3], Holland TC[2], Al-Katib A[2], Bhagwat AS[1]. [1]Wayne State University, Detroit, MI, United States, [2]Wayne State University School of Medicine, Detroit, MI, United States, [3]University of Toronto, Toronto, ON, Canada.

An overwhelming majority of human B lymphocyte malignancies derive from cells that have undergone the germinal center reaction and are associated with the expression of AID, an enzyme that converts cytosines in DNA to uracil and is required for antibody maturation through somatic hypermutation and class-switch recombination. Several studies have shown that these lymphoma cells contain a higher frequency of point mutations in many genes and chromosome translocations, but no study has yet quantified the genomic uracils, the likely direct consequence of AID expression in these cancers. We show here that human B cell lymphoma cell lines that overexpress AID accumulate unprecedented levels of uracils. These uracils are present at many loci beyond the immunoglobulin genes. The high genomic uracil levels in these tumors may result from an imbalance between expression of AID and UNG genes. These cells also contain elevated levels of other types of DNA damage including abasic sites, and single- and double-strand breaks, and have reduced viability. B-cell lymphomas continuously accumulate DNA lesions therefore compromising genomic integrity, due to the action of an endogenous agent, the enzyme AID.

P88

Studies on Bioactivation of Human Carcinogen Aristolochic Acid. Sidorenko VS[1], Attaluri S[1], Hashimoto K[1], Zaitseva I[1], Iden CR[1], Dickman KG[1,2], Moriya M[1], Johnson F[1,3], Grollman AP[1,2]. [1]Stony Brook University, Department of Pharmacological Sciences, Stony Brook, NY, United States, [2]Stony Brook University, Department of Medicine, Stony Brook, NY, United States, [3]Stony Brook University, Department of Chemistry, Stony Brook, NY, United States.

Ingestion of *Aristolochia* plants, widely used as herbal remedies throughout the world, is associated with the development of chronic kidney disease and urothelial carcinomas of the upper urinary tract (UTUC). Aristolochic acids (AAs) - naturally occurring polyaromatic nitroarenes - are responsible for these toxic events. Recently, we found high levels of mutations linked to AAs exposure in tumors of patients from Taiwan with AA-induced UTUC. AAs require bioactivation to form mutagenic aristolactam-DNA adducts (AL-DNA). Not all exposed individuals develop nephropathy and/or UTUC, implying a role for unknown genetic factors. Identifying enzymes responsible for bioactivation and variants in genes controlling their activities, may enable the identification of individuals at risk. Nitroreduction is the first necessary step in nitroarene bioactivation. N-hydroxyaristolactams (AL-NOHs) are the stable products of the partial nitroreduction of AAs. To demonstrate the critical role of sulfotransferases in the bioactivation of AL-NOHs, we employ mass spectrometry to show the sulfonation of AL-NOHs by human sulfotransferases, and $_{32}$P-postlabeling DNA adduct analysis to monitor covalent binding of synthetic or enzymatically derived AL-N-sulfooxyesters to DNA. The later technique was also utilized to investigate the impact of the inhibition of sulfotransferases on the DNA adduction mediated by AAs and AL-NOHs in human proximal tubule cell line. Thus, we conclude that AL-NOHs, arising as the products of the partial nitroreduction of AAs, serve as substrates for sulfotransferases leading to the appearance of AL-N-sulfooxyesters, which undergo solvolysis and produce highly active nitrene species that covalently bind to DNA, forming AL-DNA adducts responsible for initiating UTUC.

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Resveratrol Inhibits Oxidative Damage Induced by Arsenic Trioxide. Zhang Z[1], Chen C[1], Jiang X[1], Lai Y[1,2], Liu Y[2]. [1]Department of Environmental Health, West China School of Public Health, Sichuan University, Chengdu, Sichuan, China, [2]Department of Chemistry and Biochemistry, Florida International University, Miami, FL, United States.

Arsenic trioxide (As_2O_3) is commonly used to treat acute promyelocytic leukemia and solid tumors. However, the clinical application of the chemotherapeutic agent is limited by its cyto- and genotoxicity. Thus, relief of As_2O_3 toxicity appears to be critically important for improving As_2O_3-mediated chemotherapy. In this study, for the first time, we demonstrated a protective effect of resveratrol against As_2O_3-induced oxidative damage in normal human cells. We discovered that treatment of normal human bronchial epithelial (HBE) cells with 5 μM resveratrol for 24 h significantly reduced the production of DNA damage and chromosomal breakage as well as apoptosis induced by 20 μM As_2O_3. We demonstrated that the protective effect of resveratrol against DNA damage was correlated with a decreased level of reactive oxygen species and lipid peroxidation in HBE cells treated by As_2O_3. We further demonstrated that resveratrol protected As_2O_3 toxicity by modulating glutathione (GSH) biosynthesis, recycling and consumption, thereby altering the progression of cellular apoptotic process. This was supported by the results showing that resveratrol significantly prevented enhanced activities of caspase-3, 8 and 9 as well as high levels of Fas, Fas-L and cytochrome c induced by As_2O_3, thereby inhibiting progression of apoptosis. Our study suggests that resveratrol protects against As_2O_3-induced oxidative damage via maintenance of GSH homeostasis and inhibition of apoptotic progression in normal human lung cells.

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A Novel Maillard Reaction Product, Aminobenzoazepinoquinolinone-Derivative, Induces Genotoxicity and Preneoplastic Lesions in Mice. Wakabayashi K[1], Totsuka Y[2], Watanabe T[3], Kochi T[4], Shimizu M[4], Tanaka T[4]. [1]University of Shizuoka, Shizuoka, Japan, [2]National Cancer Center Research Institute, Tokyo, Japan, [3]Kyoto Pharmaceutical University, Kyoto, Japan, [4]Gifu University Graduate School of Medicine, Gifu, Japan.

Diabetes mellitus is a risk factor for various kinds of cancers, and the Maillard reaction is reported to be involved in diabetic complications. We recently demonstrated that a novel heterocyclic amine, 5-amino-6-hydroxy-8*H*-benzo[6,7]azepino[5,4,3-*de*]quinolin-7-one (ABAQ), is formed from glucose and L-tryptophan through the Maillard reaction under physiological conditions. ABAQ was strongly mutagenic to *Salmonella* strains with S9 mix, and its mutagenicity was comparable to that of PhIP. *In vivo* comet assay, DNA damage was induced by ABAQ in multiple organs (liver, lung and bone marrow) of male ICR mice, 3h after i.p. administration (50 mg/kg bw). ABAQ also induced micronucleated reticulocytes in mice in a dose-dependent manner at i.p. doses of 12.5, 25 and 50 mg/kg bw after 48h in the peripheral blood micronucleus test. Male *gpt* delta mice were orally administered five consecutive doses (25 or 50 mg/kg bw) of ABAQ per week for 3 weeks. The frequencies of *gpt* mutations in the liver of mice significantly increased in a dose-dependent manner, and GC→AT and AT→CG mutations were significantly higher in the ABAQ-treated group. Moreover, one week of exposure to dextran sodium sulfate after the single i.g. administration of ABAQ (200 mg/kg bw) resulted in increase of the incidence and number of high-grade dysplasia lesions in the colorectum of male ICR mice. Thus, it is suggested that ABAQ is involved in the diabetes-related cancer development. Studies on *in vivo* formation of ABAQ in diabetic model animals and diabetic patients are in progress in our laboratory.

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Induction of Aberrant Crypt Foci (ACF) in Rats By Haloacetic Acids. Kligerman AD[1], Geter DR[1,2], George MH[1], Moore TM[1], Wood CE[1], DeAngelo AB[1]. [1]US Environmental Protection Agency, Research Triangle Park, NC, United States, [2]Bayer Crop Science LP, Research Triangle Park, NC, United States.

Disinfection byproducts of chlorination such as trihalomethanes and haloacetic acids (HAAs) are some of the most prevalent contaminants that appear in municipal water supplies. Thus, large populations of humans are continually exposed to low concentrations of these potential toxicants. Previous studies have shown that some of these contaminants are weakly genotoxic in the Comet Assay and induce different types of tumors in animal carcinogenicity studies. In the studies reported here, we tested how the addition of HAAs, specifically dichloroacetic acid (DCA) [500 mg/l], bromochloroacetic acid (BCA)[500 mg/l], or dibromoacetic acid (DBA) [500 mg/l], to the drinking water of male F344 rats affected precursor lesions in the colon. In the first study colons were analyzed for aberrant crypt foci (ACF) after 52 weeks of exposure. Only BCA caused a statistically significant increase in ACF. In a follow-up study male and female F344 rats were exposed to 0, 250, 500, and 1000 mg/l BCA in drinking water for 26 and 52 weeks. ACF were significantly increased in the high dose group in males at 52 weeks and at all dose levels at both 26 and 52 weeks in the females. These findings support prior carcinogenicity studies showing that, of the three HAAs tested, only BCA induced tumors (adenomas) in the colon. The results also indicate that ACF as early as 26 weeks may serve as predictive biomarkers of later tumorigenesis. This abstract does not necessarily reflect US EPA policy.

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Evaluation of HPRT-dup-GFP Mice for *In Vivo* and *In Situ* Somatic Mutation Analyses. Noda A[1], Suemori H[2], Hirai Y[1], Hamasaki K[1], Kodama Y[1], Mitani H[3], Landes RD[1], Nakamura N[1]. [1]Radiation Effects Research Foundation, Hiroshima, Japan, [2]Kyoto University, Kyoto, Japan, [3]Tokyo University, Chiba, Japan.

For detecting and measuring frequencies of mutant cells arising *in vivo* in various tissues at whole body level, we have developed HPRT-dup-GFP mice. The mice carry a partial duplication of the HPRT structural gene in the X-chromosome, where the 3' end of the duplicate was combined with a GFP gene ORF. Losing one duplicate implies deletion of 7.8Kb sequences by intrachromosomal recombination, and causes recovery of wild-type HPRT with GFP, thereby producing fluorescent HPRT-GFP fusion proteins. Our system enables detection of somatic mutations *in situ* in various tissues, including pancreas, liver, small intestine, colon, lung, thyroid, and spleen lymphocytes. Mutations and dynamic behavior of somatic stem cells could also be monitored. However, somatic mutant frequencies varied widely among similarly treated mice. Additionally, we found "jack-pot" mutations in some tissues on rare occasions. 3Gy irradiation with X-rays induced mutations in small intestine and liver, but not in pancreas; the results were inconclusive in spleen lymphocytes, i.e., means differed and medians did not. We discuss the possible use of the mice for environmental mutagen research.

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Kras Codon 12 GAT Mutant Fractions in Mouse Lung over the Lifespan of the Animals. McKinzie PB, Pearce MG, McKim KL. National Center for Toxicological Research, Jefferson, AR, United States.

In vivo studies of the biological effects of chemical and compound exposures often use rodent models treated at standard study lengths of 28 days, 90 days, or 2 years. Data from studies of mutagenesis and potential carcinogenesis can be difficult to interpret because the normal levels of spontaneous mutations are unknown, especially for particular oncogenes at specific ages of the animal model being used. In the following study, mice (NCTR strain of C3B6F1) of various ages that approximate life-stages as well as corresponding to 28-day, 90-day, and 2-year bioassays were analyzed for spontaneous mutations of the cancer-relevant gene, Kras. The mutant fractions (MFs) of Kras codon 12 GGT to GAT in mouse lung at 4 weeks, 6 weeks, 8 weeks, 12 weeks, 21 weeks, and 85 weeks of age were determined. The data show that Kras GGT to GAT MFs were below the quantification level of 10^{-5} at 4, 6, 8, 12, and 21 weeks and averaged 1 x 10^{-5} at 85 weeks. These data show that age does not contribute to increases in the MF of Kras codon 12 GAT with the exception of a few animals in the most advanced age group (85 weeks). This suggests that in mouse lung any change in the Kras MF in other studies in mice 21 weeks or younger is due to treatment, whether it is control material or test material being administered.

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Mammary Ductal Carcinomas Contain *HRAS* G12D Mutant Subpopulations. Banda M, Myers MB, McKim KL, Parsons BL. National Center for Toxicological Research, Jefferson, AR, United States.

According to the COSMIC database, *HRAS* gene mutation was not detected in any of 490 mammary ductal carcinomas (DCs), although it is detected in other types of cancer (bladder, thyroid, and kidney). Also, increased *HRAS* expression has been associated with aggressiveness of breast cancer. Because minor mutant subpopulations have been shown to impact responses to molecularly-targeted cancer treatments, we investigated the prevalence of mutant subpopulations in DCs. Specifically, we employed Allele-specific Competitive Blocker PCR (ACB-PCR) to quantify *HRAS* G12D mutation in 10 normal breast tissues and 9 DCs. An ACB-PCR assay was developed, which can quantify levels of *HRAS* G12D mutation as low as 3 mutant alleles in a background of 300,000 wild-type alleles. Measurable levels (>10^{-5}) of *HRAS* G12D mutation were observed in all normal breast tissues and DCs. Significantly greater levels of *HRAS* G12D mutation were observed in DCs compared to normal breast, which had geometric mean mutant fractions of 1.3 x 10^{-4} and 7.3 x 10^{-5}, respectively. Importantly, none of the DCs had levels of *HRAS* G12D mutation that would be detectable by DNA sequencing and the majority of DCs had levels of *HRAS* G12D mutation greater than the upper 95% confidence interval of that present in normal breast. Thus, subpopulations of cells carrying *HRAS* somatic mutations may be more relevant to breast carcinogenesis than is currently recognized. Ongoing analyses of additional tumors, of different breast cancer subtypes, will provide further insight into the role of *HRAS* G12D mutation in breast carcinogenesis.

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***PIK3CA* H1047R Mutant Subpopulations in Normal Breast and Breast Tumors.** Myers MB, Banda M, McKim KL, Wang Y, Parsons BL. National Center for Toxicological Research, Jefferson, AR, United States.

Activating somatic mutations in *PIK3CA* are considered early, initiating events in breast cancer. *PIK3CA* is the most frequently mutated gene in breast cancer, detected in 25% and 24% of breast tumors in the COSMIC and TCGA databases, respectively. The H1047R mutation is the most frequent *PIK3CA* mutation, accounting for 35% of mutations in the gene. Because undetected mutant subpopulations have the potential to cause resistance to molecularly-targeted cancer therapies, this study investigated whether subpopulation of cells carrying *PIK3CA* or other hotspot point mutations are present in ductal carcinoma (DC). This is part of a broader study using the sensitive and quantitative ACB-PCR approach to quantify hotspot *PIK3CA* H1047R, *KRAS* G12D, *KRAS* G12V, *HRAS* G12D, and *BRAF* V600E mutations in normal breast (n=10) and DCs (n=10). The *PIK3CA* H1047R geometric mean mutant fractions (MFs) in normal breast and DCs were 8.03 x 10^{-4} and 1.14 x 10^{-3}, respectively, which was not a statistically-significant difference. In normal breast, *PIK3CA* H1047R MF significantly correlated with age, and the 5th, 25th, 50th, 75th, and 95th percentiles of *PIK3CA* H1047R MF were 1.6 x 10^{-5}, 5.58 x 10^{-5}, 5.81 x 10^{-4}, 1.178 x 10^{-2}, and 3.78 x 10^{-2}, respectively. None of the normal breast or DCs had a *PIK3CA* H1047R MFs ≥ 10^{-1} (i.e. that detectable by DNA sequencing). These results suggest unexpectedly high *PIK3CA* H1047R mutant subpopulations may contribute to the development of DC. Further research is required to determine whether *PIK3CA* H1047R mutant subpopulations found in normal breast contribute to breast cancer susceptibility.

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Evaluation of Vinyl Acetate- and Acetaldehyde-Induced *gpt* Mutations in Mouse Lung Fibroblast Cell Line, GDL-1. Chen Y[1], Shelton SD[1], Gollapudi BB[4], Albertini RJ[5], Fensterheim RJ[3], Budinsky R[2], Haber LT[7], Moore MM[6], Manjanatha MG[1]. [1]National Center for Toxicological Research, Division of Genetic and Molecular Toxicology, US FDA, Jefferson, AR, United States, [2]The Dow Chemical Co., Midland, MI, United States, [3]RegNet Environmental Services, Washington DC, United States, [4]Exponent, Center of Toxicology and Mechanistic Biology, Midland, MI, United States, [5]University of Vermont, Burlington, VT, United States, [6]Environ International Corporation, Little Rock, AR, United States, [7]Toxicology Excellence for Risk Assessment, Cincinnati, OH, United States.

Vinyl acetate monomer (VAM) induces nasal tumors in rats and oropharyngeal tumors in rats and mice. VAM has been reported to be weakly genotoxic *in vitro* and non genotoxic *in vivo*. A critical key event in VAM's rodent carcinogenicity is its carboxylesterase-dependent hydrolysis to acetaldehyde (AA), a genotoxic metabolite. The mutagenicity of VAM and AA was evaluated in a newly established *gpt* delta mouse lung fibroblast cell line (GDL1) harboring two reporter genes, *gpt* and *red/gam* (Spi$^-$) employing either horse serum (HS) or fetal bovine serum (FBS) since HS was shown to rapidly metabolize VAM to AA. VAM and AA were not mutagenic at the Spi$^-$ locus even when exposed up to 10 mM doses. Whereas, at the *gpt* locus, the MFs appeared to increase with doses up to 5 mM of VAM and AA with cytotoxicity ranging between 65-70%. Several *gpt* mutants were selected from these doses and analyzed for DNA sequence alterations. VAM or AA induced a significant shift in the mutational spectra from the control spectra ($P \leq 0.05$) and the predominant types of mutations induced by AA were G→T (45%) and A→T (12%) transversions as opposed to G→A (55%) transitions in the unexposed control cells. While VAM induced predominantly G→A (36%) transitions in GDL-1 cells in FBS, it induced a 2.5-fold increase in the G→T mutations (24%) in HS. These results suggest that AA is a mutagenic metabolite of VAM. Further studies could examine the mutagenic potential of VAM by itself in this immortalized transgenic cell line.

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Analysis of Dibenzo[*def,p*]chrysene Adduct Formation in a Transplacental Chemoprevention Model Using Stable Isotope Dilution UPLC-MS/MS. Harper TA[1,2], Morre J[3,4], Williams DE[1,2]. [1]Linus Pauling Institute, Oregon State University, Corvallis, OR, United States, [2]Superfund Research Center, Oregon State University, Corvallis, OR, United States, [3]Department of Chemistry, Oregon State University, Corvallis, OR, United States, [4]Environmental Health Sciences Center, Oregon State University, Corvallis, OR, United States.

Metabolism of dibenzo[*def,p*]chrysene (DBC) to the ultimate genotoxic metabolite, 11,12-dihydrodiol-13,14 epoxide (DBCDE), by P4501B1 results in the formation of covalent DNA-adducts believed to be the initiating event in DBC carcinogenesis. Our laboratory previously reported DBC to be a transplacental carcinogen resulting in lymphoblastic leukemia and lung carcinoma in murine offspring. Furthermore, we have demonstrated that maternal consumption of the dietary phytochemical indole-3-carbinol (I3C) reduces lung tumor burden and increases offspring survival. We hypothesize the chemoprotection conferred by I3C is due in part to decreased adduct formation in the lung. To test this hypothesis pregnant B6129SF1/J dams were fed a diet containing 500ppm I3C, 400ppm sulforaphane (SFN), or control diet on gestational day 9 through birth. A single oral dose of 15mg/kg DBC was administered to the pregnant dams on gestational day 17. Offspring and dams were euthanized at birth and lung tissue was analyzed for adduct formation using stable isotope dilution UPLC-MS/MS. Preliminary analysis reveals adduct formation in neonate and maternal tissue of all treatment groups. The (±)anti-cis-DBCDE-dA adduct was most prevalent in all 3 groups. Quantification of adduct levels will require further analysis. However, preliminary calculations suggest a trending decrease in adduct levels with I3C and SFN dietary intervention. We conclude that DBC or its metabolites are able to traverse the placenta and form detectable adducts in neonate lung, contributing to the formation of lung carcinoma after an *in utero* exposure. Supported by CA90890 and ES016465.

Author Index

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