EMGS Abstracts

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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
EMGS Abstracts
Supplement of Environmental and Molecular Mutagenesis
Journal of the Environmental Mutagenesis and Genomics Society

Volume 55, Number S1 ........................................................................................................ 2014

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Volume 55, Number S1, was posted the week of August 17, 2014.
Environmental Mutagenesis and Genomics Society 45th Annual Meeting Abstracts

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
### ANNUAL MEETING AGENDA

#### FRIDAY, SEPTEMBER 12

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Location</th>
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<tbody>
<tr>
<td>3:00 PM–6:00 PM</td>
<td>REGISTRATION</td>
<td>WEST REGISTRATION</td>
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<tr>
<td>3:00 PM–6:00 PM</td>
<td>SPEAKER READY ROOM</td>
<td>GRAND OFFICE</td>
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<tr>
<td>7:00 PM–9:30 PM</td>
<td>EXECUTIVE BOARD MEETING</td>
<td>EDELWEISS</td>
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#### SATURDAY, SEPTEMBER 13

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<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tr>
<td>8:00 AM–6:00 PM</td>
<td>REGISTRATION</td>
<td>WEST REGISTRATION</td>
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<td>8:00 AM–6:00 PM</td>
<td>SPEAKER READY ROOM OPEN</td>
<td>GRAND OFFICE</td>
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<tr>
<td>8:30 AM–11:00 AM</td>
<td>WORKSHOP Adverse Outcome Pathways</td>
<td>GRAND BALLROOM 6</td>
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<tr>
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<td>(Separate Registration Required)</td>
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<td></td>
<td>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</td>
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<tr>
<td></td>
<td>Introduction and Overview of AOPs and the Paradigm Covering Areas Such As What Are Their Uses, Construction, Linkages, and Validation/Identifying Data Gaps</td>
<td>Daniel L. Villeneuve, US Environmental Protection Agency</td>
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<td>8:30 AM</td>
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<td></td>
<td>Development, Testing, and Applying AOPs to Risk Assessment</td>
<td>Ian Cotgreave, Karolinska Institute</td>
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<td></td>
<td>Break</td>
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<td>10:10 AM</td>
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<tr>
<td></td>
<td>The AOP Wiki</td>
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<td>10:35 AM</td>
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<tr>
<td></td>
<td>AOPs in a Regulatory Framework</td>
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<td>11:00 AM–1:00 PM</td>
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<td>LUNCH ON YOUR OWN</td>
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<tr>
<td>11:00 AM–1:00 PM</td>
<td>COUNCIL MEETING 1</td>
<td>HIBISCUS</td>
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<tr>
<td>1:00 PM–4:00 PM</td>
<td>WORKSHOP Adverse Outcome Pathways (continued)</td>
<td>GRAND BALLROOM 6</td>
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<td>(Separate Registration Required)</td>
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<td></td>
<td>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</td>
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<td>Breakout Group 1—Grand Ballroom 6</td>
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<td>Mutagenic Mode-of-Action in Cancer</td>
<td>Rita Schoeny, US Environmental Protection Agency</td>
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<td>1:00 PM</td>
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<td></td>
<td>Breakout Group 2—Dogwood</td>
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<tr>
<td></td>
<td>Brainstorming How to Incorporate Epigenetics into AOPs</td>
<td>Catherine B. Klein, New York University School of Medicine</td>
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</tbody>
</table>
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**  
(First Meeting) | **EDELWEISS** |

| 4:00 PM–5:30 PM | **EMGS FORUM**  
*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**  
*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.

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.

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

8:30 AM–8:45 AM  WELCOME  GRAND BALLROOM 4

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
8:45 AM–9:45 AM  KEYNOTE SPEAKER (K1)

**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

**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

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

(Advance Registration Required)

2:00 PM–4:00 PM  POSTER SESSION 1 AND EXHIBITS

(Odd-Numbered Posters Attended)
### APPLIED SYMPOSIUM 1
**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*

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<tr>
<th>Time</th>
<th>Session</th>
<th>Title</th>
<th>Speaker/Institution</th>
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<tr>
<td>4:00 PM</td>
<td>Introduction</td>
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<tr>
<td>4:15 PM</td>
<td>S9</td>
<td>Variability: The Common Feature of Induced Genomic Instability</td>
<td>Jeffrey L. Schwartz, University of Washington</td>
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<tr>
<td>4:35 PM</td>
<td>S10</td>
<td>Radiation-Induced Adaptive and Nontargeted Effects: Modulating Factors and Potential Long-Term Health Consequences</td>
<td>Edouard I. Azzam, UMDNJ-Rutgers New Jersey Medical School</td>
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<td>4:50 PM</td>
<td>S11</td>
<td>Molecular Basis for the Lack of Genomic Instability in Bone Marrow Cells of Mice Exposed to Low-Dose Radiation</td>
<td>Kanokporn Noy Rithidech, Stony Brook University</td>
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<td>5:05 PM</td>
<td>Break</td>
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<tr>
<td>5:20 PM</td>
<td>S12</td>
<td>Health Risks and Benefits from Low Dose and Low Dose-Rate Environmental Exposures</td>
<td>Marianne Sowa, Pacific Northwest National Laboratory</td>
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<tr>
<td>5:40 PM</td>
<td>S13</td>
<td>Radiation-Induced Modification of Chemical Damage and Risk</td>
<td>Antone L. Brooks, Washington State University</td>
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<td>6:00 PM</td>
<td>S14</td>
<td>Interactions of Low-Dose Radiation and the Carcinogen Benzo[a]pyrene in A/J Mice</td>
<td>Veronica R. Bruce, Lovelace Respiratory Research Institute</td>
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<td>6:20 PM</td>
<td>Discussion</td>
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### BASIC SYMPOSIUM 1
**’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*

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<th>Time</th>
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<th>Speaker/Institution</th>
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<tr>
<td>4:00 PM</td>
<td>Introduction</td>
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<td>Arthur P. Grollman and Viktoriya Sidorenko, Stony Brook University</td>
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<td>4:05 PM</td>
<td>S15</td>
<td>Multi-’Omics Approaches to Environmental Cancer Risk Factors: A Spotlight on Exposure to Aristolochic Acid and Its Biological Consequences</td>
<td>Jiri Zavadil, International Agency for Research on Cancer</td>
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<tr>
<td>4:35 PM</td>
<td>S16</td>
<td>Application of ’Omics Technologies in Occupational and Environmental Research</td>
<td>Roel Vermeulen, Utrecht University</td>
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<td>5:05 PM</td>
<td>Break</td>
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<td>5:20 PM</td>
<td>S17</td>
<td>Incorporating Biological, Chemical, and Toxicological Knowledge into Predictive Models of Toxicity</td>
<td>Rita Schoeny, US Environmental Protection Agency</td>
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</tbody>
</table>
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
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
Leaders: Carol D. Swartz, Integrated Laboratory Systems, Inc.
and Jeffrey K. Wickliffe, Tulane University

New Technologies Special Interest Group
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
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<th>Time</th>
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<tr>
<td>9:45 AM–12:30 PM</td>
<td><strong>BASIC SYMPOSIUM 2</strong>&lt;br&gt;Environmental Exposures, Endogenous Processes, and Protective Mechanisms in Neurodegenerative Disease&lt;br&gt;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</td>
<td><strong>GRAND BALLROOM 6</strong></td>
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<td>9:45 AM</td>
<td>S24 Suppression of Somatic Expansion Delays Motor Decline in a Mouse Model of Huntington's Disease&lt;br&gt;Cynthia T. McMurray, Lawrence Berkeley Laboratory</td>
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<td>10:15 AM</td>
<td>S25 The Role of DNA Damage Resulting from Oxidative Stress and Lipid Peroxidation in Neurodegeneration in Models Deficient for Nucleotide Excision Repair&lt;br&gt;Laura J. Niedernhofer, The Scripps Research Institute</td>
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<tr>
<td>10:45 AM</td>
<td>Break</td>
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<tr>
<td>11:00 AM</td>
<td>S26 Mechanisms and Treatments for Radiation- and Chemotherapy-Induced Cognitive Dysfunction&lt;br&gt;Charles L. Limoli, University of California, Irvine</td>
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<td>11:30 AM</td>
<td>S27 Formaldehyde Exposure and Amyotrophic Lateral Sclerosis&lt;br&gt;Marc G. Weisskopf, Harvard University</td>
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<tr>
<td>12:00 Noon</td>
<td>Discussion</td>
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<tr>
<td>9:45 AM–4:00 PM</td>
<td><strong>POSTERS AND EXHIBITS OPEN</strong></td>
<td><strong>INTERNATIONAL BALLROOM</strong></td>
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<td>12:30 PM–2:00 PM</td>
<td><strong>WOMEN IN EMGS LUNCHEON</strong>&lt;br&gt;(Advance Registration Required)&lt;br&gt;Leaders: Meagan Myers, National Center for Toxicological Research, US FDA and Kristine L. Witt, National Institute of Environmental Health Sciences, NIH</td>
<td><strong>GRAND BALLROOM 1</strong></td>
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<td>12:30 PM</td>
<td>Networking and Introduction of Topic</td>
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<td>12:50 PM</td>
<td>L1 Protecting Pregnant Women and Their Babies from Seasonal and Pandemic Influenza&lt;br&gt;Sonja A. Rasmussen, Centers for Disease Control and Prevention</td>
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<td>1:35 PM</td>
<td>Discussion</td>
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<td>2:00 PM–4:00 PM</td>
<td><strong>POSTER SESSION 2 AND EXHIBITS</strong>&lt;br&gt;(Even-Numbered Posters Attended)</td>
<td><strong>INTERNATIONAL BALLROOM</strong></td>
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<td>4:00 PM–6:00 PM</td>
<td><strong>APPLIED PLATFORM SESSION 1</strong>&lt;br&gt;Chairpersons: Vasily N. Dobrovolsky, National Center for Toxicological Research, US FDA and Michael J. Plewa, University of Illinois, Urbana</td>
<td><strong>GRAND BALLROOM 4</strong></td>
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<td>4:00 PM</td>
<td>Introduction</td>
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<td>4:15 PM</td>
<td>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)&lt;br&gt;Dad A, Jeong CH, Wagner ED, Plewa MJ. University of Illinois at Urbana Champaign, IL, United States</td>
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</table>
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 MA1, Gagne R2, O'Brien JM3, Yauk CL2, Marchetti P2.
1Carleton University, Ottawa, ON, Canada, 2Health 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 RA1, Pendse S1, McMullen P1, Sun B1, Zhang Q1, Adeleye Y2, Carmichael P2, Andersen ME1. 1The Hamner Institutes for Health Sciences, Research Triangle Park, NC, United States, 2Unilever, PLC, Sharnbrook, Bedfordshire, United Kingdom

5:15 PM  5  Connecting Oxidative Damage to Epigenetic Alterations
Ding N1, DeStefano Shields C2, Sears CL3, Baylin SB2, O'Hagan HM1. 1Indiana University, Bloomington, IN, United States, 2Johns 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 S1, Williams A2, Guo CH1, Arlt VM3, Schmeiser HH4, Leingartner K2, Yauk CL2, White PA2, Halappanavar S3,1. 1University of Ottawa, Ottawa, ON, Canada, 2Health Canada, Ottawa, ON, Canada, 3King's College London, London, United Kingdom, 4German 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 Interindividuall 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 H1, Peng B2, Engler DA3, Xu X4, Xu B5, Mitra S1, Hegde ML1,2. 1Department of Radiation and Oncology, The Houston Methodist Hospital Research Institute, Houston, TX, United States, 2Neurological Institute, the Methodist Hospital, Houston, TX, United States, 3Beijing Key Laboratory of DNA Damage Response, Beijing, Beijing, China, 4Proteomics Core, The Houston Methodist Hospital Research Institute, Houston, TX, United States, 5Department 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
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<tr>
<th>Time</th>
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<tr>
<td>7:00 AM–8:30 AM</td>
<td><strong>SPECIAL INTEREST GROUP MEETINGS</strong> (Breakfast on Your Own)</td>
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<td></td>
<td>Epigenetics Special Interest Group</td>
<td>Grand Ballroom 1</td>
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<td></td>
<td>Leaders: Daneida Lizarraga; Janice M. Pluth, Lawrence Berkeley National Laboratory; and Caren Weinhouse, University of Michigan</td>
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<td>Transgenic and In Vivo Mutagenesis Special Interest Group</td>
<td>Grand Ballroom 2</td>
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<td>Leaders: Alexandra S. Long, Health Canada and Nan Mei, National Center for Toxicological Research, US FDA</td>
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<td>8:00 AM–10:00 AM</td>
<td><strong>POSTERS AND EXHIBITS OPEN</strong></td>
<td>INTERNATIONAL BALLROOM</td>
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<td>8:45 AM–9:45 AM</td>
<td><strong>EMGS AWARD LECTURE (L2)</strong></td>
<td>GRAND BALLROOM 4</td>
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<td>Personal Genomes, Clan Genomics, and the Clinical Implementation of Genome Analysis</td>
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<td>Chairpersons: Suzanne M. Morris and Barbara L. Parsons</td>
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<td>Lecturer: James R. Lupski, Baylor College of Medicine</td>
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<td>9:45 AM–12:30 PM</td>
<td><strong>APPLIED SYMPOSIUM 3</strong></td>
<td>GRAND BALLROOM 4</td>
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<td></td>
<td>Advances and Use of Transgenic Rodent Mutation Assays in Risk Assessment: Update on Recent Developments and Applications</td>
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<td>Chairpersons: Alexandra S. Long, Health Canada and Nan Mei, National Center for Toxicological Research, US FDA</td>
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<td>9:45 AM</td>
<td>S28 Historical Development of Transgenic Rodent Mutation Assays and Future Challenges</td>
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<td>Iain B. Lambert, Carleton University</td>
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<td>10:15 AM</td>
<td>S29 Advances in the Transgenic Rodent Assay for Germ Cell Mutagenicity Testing</td>
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<td>Francesco Marchetti, Health Canada</td>
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<td>10:45 AM</td>
<td>Break</td>
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<td>11:00 AM</td>
<td>S30 Development of Transgenic Hairless Albino Mice for Testing UVB-Induced Circadian Clock in the Skin</td>
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<td>Mugimane G. Manjanatha, National Center for Toxicological Research, US FDA</td>
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<td>11:30 AM</td>
<td>S31 Accumulation of Spontaneous Point Mutations and Deletions with Aging in gpt Delta Transgenic Rodents</td>
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<td>Kenichi Masumura, National Institute of Health Science, Japan</td>
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<td>12:00 Noon</td>
<td>S32 Genotoxicity Testing: In Vitro Methods Derived from Transgenic Mice</td>
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<td>Mirjam Luijten, National Institute for Public Health and the Environment (RIVM)</td>
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<td>9:45 AM–12:30 PM</td>
<td><strong>BASIC SYMPOSIUM 3</strong></td>
<td>GRAND BALLROOM 6</td>
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<tr>
<td></td>
<td>Environmental Epigenetics, Epigenotoxic Assays, and Regulation</td>
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<td>Chairpersons: Janet E. Baulch, University of California, Dana C. Dolinoy, and Caren Weinhouse, University of Michigan</td>
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<td>9:45 AM</td>
<td>S33 Regulation of Chemicals Based on Epigenetic Liability: Are We There Yet?</td>
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<td>Jay I. Goodman, Michigan State University</td>
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<td>10:15 AM</td>
<td>S34 Potential Mechanisms for the Transfer of Environmental Exposures to Epigenetic Change</td>
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<td>Mitchell Turker, Oregon Health &amp; Science University</td>
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<td>10:45 AM</td>
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<td>11:00 AM</td>
<td>S35</td>
<td>Epigenetic Assays: Updates, Benefits, and Limitations</td>
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<td>Catherine B. Klein, New York University School of Medicine</td>
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<td>11:30 AM</td>
<td>S36</td>
<td>Diverse Epigenetic Enzymes Empower Regulated Gene Expression</td>
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<td>Trevor K. Archer, National Institute of Environmental Health Sciences</td>
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<td>12:00 Noon</td>
<td>S37</td>
<td>Relationships between Mercury Exposure, DNA Methylation, and Cardiometabolic Risk Factors</td>
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<td>Jackie Goodrich, University of Michigan</td>
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<td>12:30 PM–1:30 PM</td>
<td>LUNCH ON YOUR OWN</td>
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<td>12:30 PM–1:30 PM</td>
<td>EDUCATION, STUDENT AND NEW INVESTIGATOR AFFAIRS COMMITTEE</td>
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<td>(Lunch on Your Own)</td>
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<td>1:30 PM–3:30 PM</td>
<td>APPLIED PLATFORM SESSION 2</td>
<td>GRAND BALLROOM 4</td>
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<td>Chairpersons: Julie A. Cox, University of Ottawa and Javier Revollo, National Center for Toxicological Research, US FDA</td>
<td>Presenting author is underlined.</td>
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<tr>
<td>1:30 PM</td>
<td>Introduction</td>
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<td>1:45 PM</td>
<td>15</td>
<td>Identification of Single Nucleotide Polymorphisms and Novel Genetic Anomalies in the Normalized Transcriptomes of TK6, WTK1, and NH32 Cells by Next Generation Sequencing</td>
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<td>Revollo J, Petibone D, Morris S, Ning B, Dobrovolsky V. National Center for Toxicological Research, Jefferson, AR, United States</td>
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<td>2:00 PM</td>
<td>16</td>
<td>Characterization of Primary Muta™ Mouse Hepatocytes: A Promising New In Vitro Tool for Mutagenicity Assessment</td>
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<td>Cox JA, Zwart EP, Luijten M, White PA. Department of Biology, University of Ottawa, Ottawa, ON, Canada, 2Laboratory for Health Protection Research, National Institute for Public Health and the Environment, Bilthoven, Utrecht, Netherlands, 3Mechanistic Studies Division, Environmental Health Science and Research Bureau, HECSB, Health Canada, Ottawa, ON, Canada</td>
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<td>2:15 PM</td>
<td>17</td>
<td>CometChip: Enabling Translation of DNA Damage and Repair Assays</td>
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<td>2:30 PM</td>
<td>18</td>
<td>MicroRNA-mRNA Regulatory Networks Help to Unravel Mechanisms of Response to BaP Exposure</td>
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<td>Lizzarraga D, 1,2 Gaj S, Brouers KJ, Timmermans L, Kleinjans JC, van Delft JH, 1Netherlands Toxicogenomics Centre, Maastricht University, Maastricht, Netherlands, 2School of Public Health, University of California, Berkeley, Berkeley, CA, United States</td>
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<td>2:45 PM</td>
<td>19</td>
<td>Characterization of microRNAs in Serum: A New Class of Biomarkers for Diagnosis of Parkinson Disease in Mouse Model</td>
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<td>Chigurupati S, Raymick J, Paule MG, Sarkar S. National Center for Toxicological Research, US FDA, Jefferson, AR, United States</td>
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</table>
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 AS1, Art VM2, Dertinger SD3, White PA1,4. 1Department of Biology, Faculty of Graduate and Postdoctoral Studies, University of Ottawa, Ottawa, ON, Canada, 2Analytical and Environmental Sciences Division, MRC-HPA Centre for Environment and Health, King's College London, London, United Kingdom, 3Litron Laboratories, Rochester, NY, United States, 4Mechanistic Studies Division, Environmental Health Science and Research Bureau, Health Canada, Ottawa, ON, Canada

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

1:30 PM  Introduction

1:45 PM  22  Impact of Repetitive Element Transcriptional Activation in Cocaine Addiction
Wang T1, Feng J2, Fargo D1, Santos J1, Nestler E2, Woychik R1. 1National Institute of Environmental Health Sciences, Durham, NC, United States, 2Mount 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 O1, Baccarelli A2, Byun H-M2, Guo L3, Zhong J2. 1University of Guanajuato, León Campus, Health Sciences Division, León, Guanajuato, Mexico, 2Harvard School of Public Health, Laboratory of Human Environmental Epigenomics, Boston, MA, United States, 3Key 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 DM1,2, Carvalho WF2, Melo COA1,3, Godoy FR4, Bastos RP4, Cruz AD3,4, Franco FC1, Arruda AA1. 1Universidade Federal de Goiás, Programa de Pós-Graduação em Genética e Biologia Molecular, Goiânia, Brazil, 2Universidade Federal de Goiás, Programa de Pós-Graduação em Ciências Ambientais, Goiânia, Brazil, 3Pontifícia Universidade Católica de Goiás, Mestrado em Genética, Goiânia, Brazil, 4Universidade 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

WEDNESDAY, SEPTEMBER 17

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

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

9:00 AM–11:45 AM APPLIED SYMPOSIUM 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 J1, Kaur P1, Chen H2, Countryman P1, Roushan M1, Flaherty D1, Brennan E1, Piehler J1, Riehn R1, Tao YJ2, Wang H1.  
1Physics Department, North Carolina State University, Raleigh, NC, United States, 2Department of Biochemistry and Cell Biology, Rice University, Houston, TX, United States, 3Genetics Department, North Carolina State University, Raleigh, NC, United States, 4Division 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-E1, 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)
### APPLIED SYMPOSIUM 5

**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.

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| 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                                                                             |                                                               |

### BASIC SYMPOSIUM 5

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

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| 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     | Topoisomeraseβ 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                                                                             |                                                               |

### COUNCIL MEETING 2

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–K2   Page S18
Lecture Abstracts          L1–L2   Page S18
Debate Lecture Abstracts   D1–D2   Pages S18–S19
Forum Abstracts            F1–F3   Page S19
Symposia Abstracts         S1–S56  Pages S20–S32
Platform Abstracts         1–28   Pages S33–S39
Poster Abstracts           P1–P97  Pages S40–S63
Author Index               Pages S64–S67
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.
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, 1 Knasmueller S, 2 Holland NT, 3 Bolognesi C, 4 Fenech MF, 5 Vrije Universiteit Brussel, Brussels, Belgium; 1 IRCCS San Raffaele Pisana, Rome, Italy; 2 Medical University Vienna, Vienna, Austria; 3 University of California, Berkeley, Berkeley, CA, United States; 4 National Institute for Cancer Research, Genova, Italy; 5 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 L.1,2 Board of Directors, Federation of American Societies for Experimental Biology, Bethesda, MD, United States; 1 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 AJ1, Allard P2, Somers CA3, Lawrence Berkeley National Laboratory, Berkeley CA, United States, 1University of California, Los Angeles, Los Angeles, CA, United States, 2University 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 LD1, Wilson TE2, Smith-Roe SL3. 1Massachusetts Institute of Technology, Cambridge, MA, United States, 2University of Michigan, Ann Arbor, MI, United States, 3National 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 P1, Waters MD2, Yauk CL3. 1Unilever, Bedford, United Kingdom, 2Integrated Laboratory Systems, Inc., Research Triangle Park, NC, United States, 3Health 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.
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 8:986-988). 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.

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 oogenesis 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—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.

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 β (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.

Genome Analyses of Single Human Oocytes for In Vitro Fertilization. Xie XS1,2, Tang F3, Qiao J1. 1Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA, United States, 2Biodynamic Optical Imaging Center, College of Life Sciences, Peking University, Beijing, China, 3Center 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 Mendelian diseases from either parent.
Factors and Potential Long-Term Health Consequences. Radiation-Induced Adaptive and Nontargeted Effects: Modulating...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, 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 irradiations 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 KN1, Jangiam W2, Gordon C1, Whorton E2. Department of Pathology, Stony Brook University, Stony Brook, NY, United States, 1Institute of Human Infections and Immunity, UTMB, Galveston, TX, United States.

Previously, we found that 0.05 Gy of 137Cs-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 1.0 Gy) do induce genomic instability. We will compare them to other low dose-induced phenomena, and consider how the biochemical effects of low dosed/low fluence exposures and their interactions among signaling processes that determine the outcome of such exposures. The risk of chronic-inflammation and aberrant-profiles of 5-methylcytosine 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 mutagenesis, 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.
Interactions of Low-Dose Radiation and the Carcinogen Benzo[a]pyrene in AJ/J Mice. Bruce VR1,2, Belinsky S1, Gott K3, March T3, Scott B1, Wilder J1. 1Lovelace Respiratory Research Institute, Albuquerque, NM, United States, 2University of New Mexico, Albuquerque, NM, United States, 3Independent 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 AJ 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 fractional 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 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


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.


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. dihydroxyacrolein, 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.


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.

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.
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 animal 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 aptamer 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 toxicologic properties of ONTs suggest that this relatively new therapeutic modality has a sufficient tolerability profile to support safe evaluation in numerous therapeutic indications.

What’s Next for Genetic Toxicological Testing for Oligonucleotide-Based Therapeutics? Sweder KGS, 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 toxicity testing to ensure more rapid, reliable assessment of genotoxicity?
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 H1, Harris P2, Williams P3, Lee D4,5,6, Pahke J7, Szczesny B8, Mita S2, Acededo-Torres K9, Ayala-Pena S10.

This work describes the use of a novel mouse model in which toxicity arising from an inherited and somatic expansion can be measured independently with 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(+/−) mice with OGG1(+/-) progeny that express the expanded full-length mutant HD allele. 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. The mice also accumulate cyclopurine lesions in the liver and kidney of HdhQ150(-/-) mice, correlating with their lack of somatic expansion in the HdhQ150(-/-) mouse line. 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.


The Scripps Research Institute, Department of Metabolism and Aging, Jupiter, FL, United States. 1University of Pittsburgh Cancer Institute, Pittsburgh, PA, United States. 2University 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, dystrophy and ataxia. Ercc1−/− 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 Ercc1−/− mice offer a promising model for neurodegeneration caused by defects in NER. We measured cyclopurine lesions accumulate in the liver and kidney of Ercc1−/− 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 Ercc1−/− 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 C1, Panifar V2, Tran K, Craver B3, Chmielewski N4, Baulch J5, Acharya M6, 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 hippocampus 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.
Subsequently, a variety of TGR models have been developed, of which containing the bacterial lacZ gene, into the mouse chromosome. Approximately 25 years ago, the first transgenic rodent (TGR) mutation model was developed by stably integrating a phage vector, 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 genotox 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.


There is a rekindled interest in the characterization of genotoxic effects of environmental agents on germ cells and the identification of human germ cell mutants. 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 mutants, 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.

Development of a Transgenic, Hairless Albino Mouse Model for Testing UV-induced Circadian Clock in the Skin. Manjanatha MG‡, Shelton SD†, Gaddamreddi S‡, Sancar A‡, Boudreau M†. National Center for Toxicological Research, Divisions of Genetic and Molecular Toxicology and Biochemical Toxicology, US FDA, Jefferson, AR, United States, ‡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/CIE/cm². 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/CIE/cm² induced significant increases (10-15-fold) in the gpt MFs over background (22 ± 3 X 10⁻⁶) and induced significant increases (4-6-fold) in the Spi MFs over background (B ± 1.5 X 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.

Accumulation of Spontaneous Point Mutations and Deletions with Aging in gpt Delta Transgenic Rodents. Masumura K, Toyoda-Hokaiwado N, Osugi N, Homma M, Ishi Y, Umemura T, Nishikawa A, Nomhi T, Division of Genetics and Mutagenesis, National Institute of Health Sciences, Tokyo, Japan, Division of Pathology, National Institute of Health Sciences, Tokyo, Japan, 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 tests, 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.
Environmental Mutagenesis and Genomics Society 45th Annual Meeting Abstracts

S32

1National Institute for Public Health and the Environment, Centre for Health Protection, Bilthoven, Netherlands, 2Leiden University Medical Center, Department of Toxicogenetics, Leiden, Netherlands, 3Health 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 Sip-) 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 glycosylase. 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. Turkcer MS, Lu Y, Glazer PM, Raber J, Impey S.
1Oregon Health & Science University, Portland, OR, United States, 2Yale 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 tel-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.
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. 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


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 J1, Moffat I1, Swartz CI2, Williams A1, Hyduke D3, Recio L1, Fornace Jr A4, Li H1, Aubrecht J5, Health Canada, Ottawa, ON, Canada, 1Integrated Laboratory Systems, Inc., Research Triangle Park, NC, United States, 2Utah State University, Logan, UT, United States, 3Georgetown University, Washington DC, United States, 4Pfizer, 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 B1, AFB1) 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 AFB1 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.
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 points-of-departure. Importantly, transcriptional BMDs for even the most sensitive pathway were on average less than three-fold relevant disease pathogenesis. Dose-related changes in gene expression were also with published risk assessments. In both studies, histological changes showed a high degree of correlation with apical responses for specific 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 L1,2, Phillips K3, Phillips S4, Swartz C, Hobbs C1. ILS, Research Triangle Park, NC, United States, 3ILS 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.

S43 Watching Base Excision Repair Glycosylases Scan for Damage. Wallace S5, 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 glycosylase’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 J1, Kaur P2, Chen H2, Riehn R3, Lin J1,3, Chen H4,4, Flaherty D1, Brennan E1, You C1, Piehler J1, Riehn R3, Tao YJ1, Wang H1, 1Physics Department, North Carolina State University, Raleigh, NC, United States, 2Department of Biochemistry and Cell Biology, Rice University, Houston, TX, United States, 3Genetics Department, North Carolina State University, Raleigh, NC, United States, 4Division 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 prefer 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. Opreško 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.


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

S47 Watching Nucleotide Excision Repair Proteins: One Molecule at a Time. Van Houten B, Kong M, Liu L, Ghodke H, Kad N, Min J-H, Watkins S. Department of Pharmacology and Chemical Biology, University of Pittsburgh School of Medicine and University of Pittsburgh Cancer Institute, Pittsburgh, PA, United States; Department of Biophysics, University of Kent, Canterbury, United Kingdom; Department of Chemistry, University of Illinois at Chicago, Chicago, IL, United States; 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 tethropes, 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.


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 ubiquitination. Within a dedicated DNA damage-induced proteomic-ubiquitlation 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.
Environmental Mutagenesis and Genomics Society 45th Annual Meeting Abstracts

S49

Webster AL,1,2 Williams A,1 Recio L,1 Yauk CL.1 1Health Canada, Ottawa, ON, Canada; 2Carleton University, Ottawa, ON, Canada. 1ILS, 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 histopathological 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 subchronically 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-H1, Hyduke DR2, Yauk CL, Aubrecht J, Fornace Jr. AJ. 1Georgetown University, Washington, DC, United States; 2Pfizer Inc., Groton, CT, United States; 3Utah State University, Logan, UT, United States; 4Health 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 shrunk centroids (NSC) algorithm. TGx28-65 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,1,2 Wang C,1,2 Gong D,1 Thierry-Mieg J1, Thierry-Mieg D,1 Xu J, Fang H, Kreil DP3, Megherbi D, Li J, Paules RS1, Shi L1, Auerbach SS1, Tong W2. 1National Institute of Environmental Health Sciences, Research Triangle Park, NC, United States; 2National Center for Toxicological Research, Jefferson, AR, United States; 3Loma Linda University, Loma Linda, CA, United States; 4National Center for Biotechnology Information, Bethesda, MD, United States; 5University of California, San Diego, CA, United States; 6Boku University, Vienna, Austria; 7University of Warwick, Coventry, United Kingdom; 8University of Massachusetts, Lowell, MA, United States; 9Kelly 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 the innovative bioinformatics 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. We have used coexpressed gene network analysis (WGCNA) to allow an unbiased 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 response modules representing sexually dimorphic function, stress response and DNA damage pathways are present and biologically relevant. Based on their transcriptional 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.
S32 Environmental Mutagenesis and Genomics Society 45th Annual Meeting Abstracts

S55 Topoisomerase IIβ Is Involved in Initiation of Interstrand Cross-Link Repair in Mammals. Smeaton MB, Bell EH, Austin CA, Deteding LJ, Toner KB, Hanawalt PC, Miller PS. 1Department of Biochemistry and Molecular Biology, Johns Hopkins School of Public Health, Baltimore, MD, United States, 2Department of Biology, Stanford University, Stanford, CA, United States, 3Institute for Cell and Molecular Biosciences, Newcastle University, Newcastle upon Tyne, United Kingdom, 4Laboratory of Structural Biology, NIH, Research Triangle Park, NC, United States.

Interstrand cross-links (ICLs) are a type of DNA damage that covalently bind the opposing strands of the DNA, and 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 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 topol catalytic inhibitor, merbarone, was able to abolish the unhooking activity in extracts. Humans have two isoforms of topol, α and β. Recombinant topolβ, 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 topolβ 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.

S54 Genome Instability Induced by Eukaryotic Topoisomerase II. Nitisss 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.

S33 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 adding the protein. Polyphenols often can be activated to quinones in the presence of dietary topoisomerase II poisons incorporate reactive groups such as quinones (thymoquinone) and act by covalently adding the protein. Polyphenols often can be activated to quinones in the presence of

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 fluorquinolones 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 block the clastogenicity of classical 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 benzimidaolizes 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 Trihalocetic Acids Water Disinfection By-Products (DBPs). Dad A, Jeong CH, Wagner ED, Plewa MJ. University of Illinois at Urbana, Champaign, IL, United States.

The haloacetic acids (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 dehydrogenase 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 IacZ Mutations in the Bone Marrow and Sperm of Muta Mouse Males Using Next-Generation Sequencing. Beal MA1, Gagne R2, O’Brien JM1, Yauck CL2, Marchetti F2. 1Carleton University, Ottawa, ON, Canada; 2Health 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 3,589 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.
Connecting Oxidative Damage to Epigenetic Alterations, Ding N1, DeStefano Shields C2, Sears CL2, Baylin SB2, O’Hagan HM1. Indiana University, Bloomington, IN, United States, 1Johns 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.

Large Transcription Units Are Hotspots for Copy Number Variants Induced by Replication Stress, Glover TW, Artl MF, Park SH, Rajendran S, Paulson M, Lijnman 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 hotspots 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 Mbp 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.


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.


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.
Tissue-Specific Transcriptomic Analysis Undermines the Utility of Benzo(a)pyrene As a Point of Reference for Carcinogenic Polycyclic Aromatic Hydrocarbons. Labib S, Williams A, Guo CH, Arti VM. Schmeiser HH, Leinhardt K, Yauk CL, White PA, Halapannavar S.

University of Ottawa, Ottawa, ON, Canada, 2Health Canada, Ottawa, ON, Canada, 3King’s College London, London, United Kingdom, 4German 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, immunoinflammatory response, and cell signaling 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.


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 overhanging 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 Dn14, 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 overhanging polarity at genomic DSBs influence end-processing and the course of mutagenesis.
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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 longevity 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 a dominant bacterial strain in the health beneficial microbiota. Just this strain 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.

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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, Zwart EP, Luijten M, White PA. 1Department of Biology, University of Ottawa, Ottawa, ON, Canada, 2Laboratory for Health Protection Research, National Institute for Public Health and the Environment, Bilthoven, Utrecht, Netherlands, 3Mechanistic 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, 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-methylumbelliferylone/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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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 D1,2, Gaj S1, Brauers KJ1, Timmermans L1, Kleinjans JC1, van Delft JH2. Netherlands Toxicogenomics Centre, Maastricht University, Maastricht, Netherlands, 1School 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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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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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.
Multi-Endpoint Comparison of Low-Dose Responses to Benzo(a)pyrene. Long AS,1 Arlt VM,2 Derington SD,2 White PA.2 1Department of Biology, Faculty of Graduate and Postdoctoral Studies, University of Ottawa, Ottawa, ON, Canada, 2Analytical and Environmental Sciences Division, MRC-HPA Centre for Environment and Health, King’s College London, London, United Kingdom, 3Litron Laboratories, Rochester, NY, United States. 4Mechanistic 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., BMDL01) values were calculated for each endpoint. The lowest BMDL01 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.

Impact of Repetitive Element Transcriptional Activation in Cocaine Addiction. Wang T,1 Feng J,1 Fargo D,1 Santos J,1 Nestler E,2 Woychik R.1 1National Institute of Environmental Health Sciences, Durham, NC, United States, 2Mount 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 ~80% 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.

Topoisomerase Ila 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,2 Zhong J.1 1University of Guanajuato, León Campus, Health Sciences Division, León, Guanajuato, Mexico, 2Harvard School of Public Health, Laboratory of Human Environmental Epigenomics, Boston, MA, United States, 3Key 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 Ila (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.

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 13C, 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.
Evaluating Genomic Damages and GSTM1 and GSTT1 Polymorphisms in Rural Workers Occupationaly Exposed to Pesticides: A Case-Control Study in an Agropastoral Brazilian State. Silva DM, Carvalho WF, Melo COA, Godoy FR, Bastos RR, Cruz AD, Franco FC, Arruda AA, Universidade Federal de Goiás, Programa de Pós-Graduação em Genética e Biologia Molecular, Goiânia, Brazil, Universidade Federal de Goiás, Programa de Pós-Graduação em Ciências Ambientais, Goiânia, Brazil, Pontificia Universidade Católica de Goiás, Mestrado em Genética, Goiânia, Brazil, 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.

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=0.0005), 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.

PON1 As a Model for Integration of Genetic, Epigenetic, and Expression Data on Candidate Susceptibility Genes. K, Yousef S, 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, shells, and islands in newborns and 9-year-old children (Illumina 450K Methylation BeadChip) and observed significant decreases in arylerase 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 arylerase activity. Our data suggest both genetics and epigenetics contribute to PON1 variability and thus potential susceptibility to adverse neurodevelopment outcomes.

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 Caenhabditis 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 these features are shared between BPA and BPS, we also found distinct
P1

Abstract Withdrawn.

P2

In Vitro Assessment of Genotoxic Effects of Ginkgo Biloba Leaf Extract and Its Eight Constitutes. Mei N1, Lin H1, Guo X1, Manjanatha MG1, Moore MM2. 1National Center for Toxicological Research, Jefferson, AR, United States, 2ENVIRON 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


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 were TA98, TA100, TA1535, TA1537 and WP2 uvrA. 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 compiled 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 increasing 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 smoke 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 ≤ 0.05). In TA1537 overall, there were no differences between the EC and CC. Significant decreases in micronuclei induction (p ≤ 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 in 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 MAD1, Marcondes JPC2, Prado RP3, Andrade PFB1, Luperini BCO1, Rudge MVC3, Salvadori DMF1. 1OMICS, Laboratory of Toxicogenomics & Nutrigenomics, Department of Pathology, Botucatu Medical School, UNESP, São Paulo State University, Botucatu, SP, Brazil, 2Department 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) 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.
Comparison of JAK1 Inhibitors Using Whole Blood and Isolated Human Peripheral Blood Lymphocytes in the In Vitro Micronucleus Test. Hurldado SB 1, Moy ML 1, Wells MM 1, Harstad EB 2, Farabaugh CS 1.

The University of Findlay, College of Pharmacy, Findlay, OH, 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, purity or metabolite testing.


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 lymphocytes 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 aim 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.

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.

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 cell 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 (H2O2).


Validation of these assays includes the ability to accurately detect chemicals that are not genotoxic themselves, but are converted into mutagenic and carcinogen 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 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.

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 3, Pfuhler S 4. Procter and Gamble Co, Mason, OH, United States, 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 carcinogen 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 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 K1, Bruce SW1, Springer S1, Lawlor TE1, Aardema MJ1. 1BioReliance by SAFC, Rockville, MD, United States, 2Marilyn 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 it is 18.34 ± 7.20. These data are routinely used to compare to patient samples. Result 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 B1, Ross S1, Trask J1, Carmichael PL1, Adeleye Y1, Andersen ME1, Clewell R1. 1The Hamner Institutes for Health Sciences, Durham, NC, United States, 2Unilever, 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), H2O2, (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 K1,2, Swenberg J1, Nakamura J1. 1Department of Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, NC, United States, 2Drug 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 O6 position of guanine, leading to the formation of O6-alkylguanine. Because the O6-alkylguanine adducts are suspected to be repaired by O6-methylguanine-DNA methyltransferase (MGMT), we addressed the effects of an MGMT inhibitor, O6-benzylguanine (O6BG), on the DNA damage response by IPMS, MMS, or EMS. While O6BG potentiated the DNA damage response to MMS and EMS in FancD2-deficient cells, IPMS caused a similar extent of DNA damage response regardless of O6BG. These results strongly suggest that MGMT can repair both O6-methylguanine and O6-ethylyguanine adducts but not O6-isopropylguanine adducts. Although these GIs are unknown to cause DNA-DNA or DNA-protein crosslinks, all O6-alkylguanines investigated in this study were subject to a FancD2-mediated DNA repair pathway.


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. Golbakhvari N1, Golbakhvari A2, Benfenati E3, Cronin M4, Rasulev B5, Leszczynski J6, Mario Negri Institute for Pharmaceutical Research, Milan, Italy, 2Jackson State University, Jackson, MS, United States, 3Liverpool 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 DM1, Ding W2, Mustafa T3, Lafont A4, Xu Y5, Watanabe F6, Casciano DT7, Dobrovolsky V8, Biris AS5, Morris SM9. 1Division of Genetic and Molecular Toxicology, National Center for Toxicological Research, Jefferson, AR, United States, 2Center for Integrative Nanotechnology Sciences, University of Arkansas at Little Rock, Little Rock, AR, United States, 3Biology Department, College of Science for Women, University of Baghdad, Baghdad, 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, EndoII 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 EndoII 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 B1 Reveals Significance of DNA Damage Tolerance Genes in Conferring Toxin Resistance. Fasullo MT1, Cera CA1, Bard J2, Freedland J1, Egner P3, Begley T4, 1State University of New York, Albany, NY, United States, 2State University of New York at Buffalo, Buffalo, NY, United States, 3Johns Hopkins University, Baltimore, MD, United States.

The mycotoxin aflatoxin B1 (AFB1) is the most potent liver carcinogen. A signature p53 mutation is found in AFB1-associated hepatic tumors, suggesting that AFB1 is a potent genotoxin. P450 enzymes convert AFB1 into a highly reactive epoxide that forms N7-guanine DNA adducts. In CYP1A2-expressing budding yeast, AFB1 is a weak mutagen but a potent recombining agent, 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 AFB1 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 AFB1 for 20 hrs. We identified genes that confer resistance to AFB1 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.
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


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 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


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 (anogen) 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


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 μg/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


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


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? Arovo 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, reproductive 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). Three 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. Attab 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 C1, Avlasevich SL1, Carlson KM1, Torous DK2, Bemis JC2, MacGregor JT2, Dertinger SD1. 1Litron Laboratories, Rochester, NY, United States, 2Toxicological 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<sup>N</sup>-CD59<sup>-</sup>), or mutant phenotype erythrocytes (RBC<sub>CD59</sub><sup>-</sup>). 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 (MutalFlow<sup>®</sup>) to determine the mutant frequency. Additionally, samples collected on day 4 were analyzed for micronucleated reticulocyte (MN-RET) frequency (MicroFlow<sup>®</sup>). %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<sup>N</sup>-CD59<sup>-</sup> and RBC<sub>CD59</sub><sup>-</sup> frequencies in both sexes. At the high dose level, the absolute RET<sup>N</sup>-CD59<sup>-</sup> and RBC<sub>CD59</sub><sup>-</sup> frequencies were somewhat lower for females compared to males. There was a significant Sex X Treatment effect on RET<sup>N</sup>-CD59<sup>-</sup> but not RBC<sub>CD59</sub><sup>-</sup> frequency. The highest two doses caused similarly elevated %MN-RET frequencies. Although there were quantitative differences in RET and RET<sup>N</sup>-CD59<sup>-</sup> 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. Elhoujji 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<sup>-</sup>negative reticulocytes (RETCD59<sup>-</sup>) and erythrocytes (RBCCD59<sup>-</sup>) 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<sup>-</sup> and RBCCD59<sup>-</sup> frequencies in their respective studies in both 3 day and 28 day studies. The RETCD59<sup>-</sup> and RBCCD59<sup>-</sup> 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.


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.


Introduction: Barrett’s Esophagus (BE) is a premalignant condition affecting the lower esophageal mucosa, caused by chronic Gastro-Esophageal Reflux 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.
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 in 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 frequency, 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 leucocyte 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.

P33 Evaluation and Comparison of Big Blue® Mouse and Rat Transgenic Rodent (TGR) Mutation Assays with N-Ethyl-N-Nitosourea (ENU) And Benzo(a)pyrene (BaP). Young RR1, Dinesdurage H1, Elbecki RH1, Bruning D2, Lawlor TE2, Aardema MJ1,2. BioReliability by SAFC, Rockville, MD, United States, 1Marilyn 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 B6CF1) 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±6.2x10-6 in mice and 43.4±6.8x10-6 in rats. Bone marrow background mutant frequency was 38.1±14.2x10-6 in mice and 29.7±14.3x10-6 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 RR1, Dinesdurage H1, Stankowski LF1, Kulkami R1, Lawlor TE1, McKeon M1, Xu Y1, Bruning D2, Avlasevich S2, Torous DK2, Dertinger SD2, Aardema MJ1,2. BioReliability by SAFC, Rockville, MD, United States, 1Litron Laboratories, Rochester, NY, United States, 2Marilyn 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 (RETcII) 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. Significant increases (p<0.001) in cII 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). Peripheral blood was collected ~3 hours before sacrifice and analyzed for Pig-a mutant phenotype and micronuclear frequencies by flow cytometry using MutaFlow® and MicroFlow® kits, respectively (Litron). BaP induced significant increases (p<0.05) in RETcII, ENU, and mnRET (61.1-, 12.2-, and 3.8-fold, respectively). ENU induced significant increases (p=0.001) in RETcII and RBCcII, (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 mnNCE or mnRET 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 micronucleus measurements can easily be integrated. Attention to timing between dosing and blood collection is important for optimal micronuclei detection.
**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-controllers, 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 (BaP) 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 BaP. 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 BaP 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, Simon BR, Dertinger SD, Torous DK, Avlasevich SL, Wickliffe JK. 1Tulane University, New Orleans, LA, United States, 2Liton 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 micronuclear 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. Micronuclear 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, Pinto IP, Franco FC, da Silva JF, Godoy FR, Melo COAT, Silva DM, da Silva CC, da Cruz AD, Pereira RW. 1Universidade Católica de Brasília, Brasília, Distrito Federal, Brazil, 2Pontifícia Universidade Católica de Goiás, Goiânia, Goiás, Brazil, 3Universidade 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.
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, gtk, glut4, insr, irs1, pp1r3c, pamlp, socs2, and sreb1 was determined by qRTTPCR 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 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.
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.

Transcriptional Alterations in the SOD2 Gene Is Not Related to the Newborn Obesity Predisposition. Marcondes JPC, Lidermedicine MAD, Andrade FPF, Luperino BCO, Rudge MVC, Salvadori DMF. Department of Pathology, Botucatu Medical School, São Paulo State University, UNESP, Botucatu, São Paulo, Brazil, 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 comorbidities. 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.

High-Resolution Genotyping Arrays Document Genetic Variation at Nonsynonymous SNPs Providing Insight into Environmentally-Responsive Genes. Milojevic M, Charron B, Edge AK, Eltutis 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 nonsynonymous SNP list. For the entire nonsynonymous 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.

Micronutrient Supplementation and Genetic Damage in Obese Brazilian Women. Luperini Bruno CO, Campos Joara P, Marcondes Paulo C, Prado Renato P, Novaia Patricia SF, Oliveira Maria Rita M, Salvadori Daisy MF. Botucatu Medical School, UNESP, São Paulo State University, Botucatu, Sao Paulo, Brazil, 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 = 45.69 ± 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 micronuclear 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).
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.


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.

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.


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.
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 24h. We therefore chose to study the hsa-mir-770-5p target gene Statm1 (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 24h 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 immunohistochemical (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.

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 Dichroism. 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.
High-Resolution SNP Genotyping Offers a Novel Genome-Wide Approach for Analysis of the Spatial Distribution of Mutations. Beaver JM, 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, thundershovers 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.

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.


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 GAG and AGG 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 β 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.


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. Cyclodeoxyuridine 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 β 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-cyclo-da 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.
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MSH2-MSH3 Promotes GAA Repeat Expansion by Stimulating DNA Polymerase β Activity during Base Excision Repair. Liu Y1, Beaver JM1, Chan NLS2, Zhang Z1, McMurray CT1, Liu Y1. Florida International University, Miami, FL, United States, 1Lawrence Berkeley National Laboratory, Berkeley, CA, United States, 2Sichuan University, Chengdu, China.

Expansion of GAA-TC 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-TC 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-TC repeats. In this study, we discovered that MSH2-MSH3 complex suppressed GAA repeat deletions during base excision repair (BER) in the context of (GAA)n 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 strand 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 repeats, 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 Y1, Wu X1, 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 showed 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.

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Molecular Signature and DNA Damage Pathways Altered in Male Largemouth Bass (Micropterus salmoides) By a Single Dose of Benzene and Trichloroethylene. Colli-Dula RC1, Mehino AC2, Kroll KJ1, Barber DS1, Vulpe CD1, Denslow ND1. 1University of Florida, Gainesville, FL, United States, 2Southern California Coastal Water Research Project, Costa Mesa, CA, United States, 3University of California, Berkeley, 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.

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A 5′-deoxy-8-purine Lesion Induces Genome Instability via DNA Polymerase β during DNA Replication and Base Excision Repair. Liu Y1, Xu M1, Jiang Z1, Terzidis M1, Masi A2, Chatgilialoglu C1. 1Department of Chemistry and Biochemistry, Florida International University, Miami, FL, United States, 2I.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 cyclopyrimidine lesions resulting in mutagenesis. Furthermore characterization of the roles of cyclo-dAs on CTG repeat deletion 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.
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, Lindeman TE, Shockley ME, Keshava C, Weston A, Poirier MC. National Cancer Institute, Bethesda, MD, 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 CYP1A1 and CYP1B1, genes which code for metabolic enzymes that form r7, 18, 19-trihydroxy-c-10-(N2-deoxyguanosyl)-7,8,9,10-tetrahydrobenzo[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 4133-5707 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/10^6 nucleotides while MCF-7 cells had 790 adducts. In the NHMECs, a linear association (p=0.0015) was observed between BPUG 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.

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 chloroacetalddehyde (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.


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 H2O2 and identify DNA repair pathways that may affect OS induced susceptibility to mutagenesis. Low concentrations of H2O2 actually found in cells during inflammatory processes were taken. Interestingly, DNA Damage Repair responses in DT40 cells, using reverse genetic approach, revealed hypersensitivity of Rad54, Rad51c, XRCC2, Ku70 and Lig IV deficient cells to H2O2, indicating the potential role of DSBs in H2O2 toxicity. High levels of 8-oxo-dG lesions and OCDLs measured with a modified PFGE version were also found in H2O2 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.

BPA Modulates Repair of Oxidative DNA Damage by Base Excision Repair Pathway. Gassman NR, Stefaniack 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-dG, 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 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.
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 H2O2 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 H2O2 (50% reduction, p<0.05), suggesting that miR155 may play a key role in inhibition of polB and promotion of senescence.

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Regulation of Base Excision Repair in Genome Maintenance.  
Limbpole 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.

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A Human Short ORF-Encoded Peptide That Interacts with Ku and Stimulates Nonhomologous End Joining.  
Hanakahi LA1, Slavoff SA2, Heo J, Budnik BA3, Saghatelian A3. University of Illinois, Rockford, IL, United States, 1Harvard 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 break repair by NHEJ and highlights the potential biological relevance of SEPs in the DNA damage response and in DNA repair.
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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 3methyladenine (3MeA) and O^2^-methylguanine (O^2^-MeG). 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-mediated protection model.

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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. ^1Central China Normal University, Wuhan, Hubei, China, ^2University 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-5-mercapto-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 and 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.

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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, Florida A&M University, Tallahassee, FL, United States, ^1Bethune-Cookman University, Daytona Beach, FL, United States.

Cadmium Chloride (CdCl2) 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 CdCl2 plus NAC. The results showed that cells viability was significantly decreased to 44.5% in the cells treated with CdCl2 alone in comparison to the untreated control cells (100%), NAC effectively ameliorated the CdCl2 adverse effect of cell viability. The cytokines expression measured by Ray Biotech arrays and confirmed by the ELISA showed differential expression patterns. In the CdCl2 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, Fil-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 CdCl2 alone. The cytokines that were down regulated in the CdCl2 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 CdCl2 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.

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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’-dichlorodiphenyldichloroethylene (p,p’-DDE), the major metabolite of dichlorodiphenylichloroethane (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^-^-^-^-; 2) 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.
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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.

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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 development 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 tests. 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.

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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.

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Establishment and Use of a Cell Line for High-Throughput Screening of DNA Demethylating Agents. Yoshikazu 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 (pCIG) was heavily methylated, and that was re-expressed sensitively in response to a DNA demethylating agent, 5-aza-2'-deoxycytidine (5-aza-dC). Then, EGFP-RES-LUC marker genes connected downstream of the pCIG 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.
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 dinucleotides 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.


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.

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

Studies on Bioactivation of Human Carcinogen Aristolochic Acid. Sidorenko VS 1, Attaluri S 2, Hashimoto K 1, Zaitseva I 1, Iden CR 1, Dickman KG 2, Moriya M 3, Johnson F 1,2, Grollman AP 1,2. Stony Brook University, Department of Pharmacological Sciences, Stony Brook, NY, United States, 2Stony Brook University, Department of Medicine, Stony Brook, NY, United States, 3Stony 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 γP-postlabeling DNA adduct analysis to monitor covalent binding of synthetic or enzymatically derived AL-N-sulfonoxyster 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-sulfonoxysters, which undergo solvolysis and produce highly active nitrene species that covalently bind to DNA, forming AL-DNA adducts responsible for initiating UTUC.
Resveratrol Inhibits Oxidative Damage Induced by Arsenic Trioxide. 
Zhang Z1, Chen C1, Jiang X1, Lai Y2, Liu Y1. 1Department of Environmental Health, West China School of Public Health, Sichuan University, Chengdu, Sichuan, China, 2Department of Chemistry and Biochemistry, Florida International University, Miami, FL, United States.

Arsenic trioxide (As2O3) is commonly used to treat acute promyelocytic leukemia and solid tumors. However, the clinical application of the chemotherapeutic agent is limited by its cytotoxicity. Thus, relief of As2O3 toxicity appears to be critically important for improving As2O3-mediated chemotherapy. In this study, for the first time, we demonstrated a protective effect of resveratrol against As2O3-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 As2O3. 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 As2O3. We further demonstrated that resveratrol protected As2O3 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 increased activities of caspase-3, 8 and 9 as well as high levels of Fas, Fas-L and cytochrome c induced by As2O3, thereby inhibiting progression of apoptosis. Our study suggests that resveratrol protects against As2O3-induced oxidative damage via maintenance of GSH homeostasis and inhibition of apoptotic progression in normal human lung cells.

A Novel Maillard Reaction Product, Aminobenzoazepinoquinolinone-Derivative, Induces Genotoxicity and Preneoplastic Lesions in Mice. 
Wakabayashi K1, Totsuka Y2, Watanebe T1, Kochi T1, Shimizu M1, Tanaka T1. 1University of Shizuoka, Shizuoka, Japan, 2National Cancer Center Research Institute, Tokyo, Japan, 3Kyoto Pharmaceutical University, Kyoto, Japan, 4Gifu 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-8H-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—TG 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.

Induction of Aberrant Crypt Foci (ACF) in Rats By Haloacetic Acids. 
Kliegerman AD1, Geter DR2, George MH1, Moore TM1, Wood CE1, DeAngelo AB3. 1US Environmental Protection Agency, Research Triangle Park, NC, United States, 2Bayer 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 carcinogens. 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 demonstrated that 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 colonies 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 28 weeks may serve as predictive biomarkers of later tumorigenesis. This abstract does not necessarily reflect USEPA policy.

Evaluation of HPRT-dup-GFP Mice for In Vivo and In S itu Somatic Mutation Analyses. 
Noda A1, Suemori H1, Hirai Y1, Hamasaki K1, Kodama Y2, Mitani H2, Landes RD3, Nakamura N1. 1Radiation Effects Research Foundation, Hiroshima, Japan, 2Kyoto University, Kyoto, Japan, 3Tokyo 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. 3 Gy 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.
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 95 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 95 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 (95 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.


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.


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^{-5} and 1.4 x 10^{-4}, 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^{-5}, 1.178 x 10^{-4}, and 3.78 x 10^{-4}, respectively. None of the normal breast or DCs had a PIK3CA H1047R MF ≥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.

Evaluation of Vinyl Acetate- and Acetaldehyde-Induced gpt Mutations in Mouse Lung Fibroblast Cell Line, GDL-1. Chen Y, Shelton SD, Gollapudi BB, Albertini RJ, Fensterheim RJ, Budinsky R, Haber L, Moore MM, Manjanatha MG. 1National Center for Toxicological Research, Division of Genetic and Molecular Toxicology, US FDA, Jefferson, AR, United States, 2The Dow Chemical Co., Midland, MI, United States, 3RegNet Environmental Services, Washington DC, United States, 4Exponent, Center of Toxicology and Mechanistic Biology, Midland, MI, United States, 5University of Vermont, Burlington, VT, United States, 6Environ International Corporation, Little Rock, AR, United States, 7Toxicology 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 (GDL-1) 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 ≤ 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.
Analysis of Dibenzo[def,p]chrysene Adduct Formation in a Transplacental Chemoprevention Model Using Stable Isotope Dilution UPLC-MS/MS. Harper TA\textsuperscript{1,2}, Morre J\textsuperscript{3,4}, Williams DE\textsuperscript{1,2}. \textsuperscript{1}Linus Pauling Institute, Oregon State University, Corvallis, OR, United States, \textsuperscript{2}Superfund Research Center, Oregon State University, Corvallis, OR, United States, \textsuperscript{3}Department of Chemistry, Oregon State University, Corvallis, OR, United States, \textsuperscript{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.
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Electronic Office: Dr. Francesco Marchetti
Mechanistic Studies Division, Environmental Health Science and Research Bureau
Healthy Environments & Consumer Safety Branch, Health Canada, 50 Columbus Drive, Ottawa, Ontario, K1A 0K9 Canada
Telephone: (613) 957-1337, Telefax: (613) 941-8530
E-mail: Francesco.marchetti@hc-sc.gc.ca

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