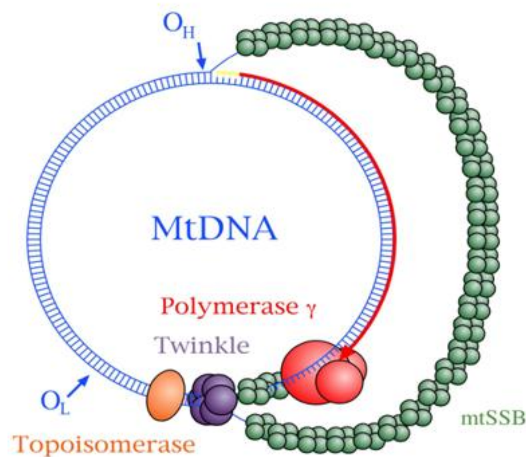
Genetics and Environmental Mutagenesis Society

34th Annual Fall Meeting

Mitochondrial DNA Mutagenesis and Human Health Impacts



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CONTENTS

Cover Page -- **1**

2015 GEMS Officers-- **2**

President's Message -- **3**

Program Agenda -- **4**

Bio-sketches and Abstracts of Invited Speakers -- **5-6**

Invited Talks Abstracts--- **7-8**

Short Talk Abstracts --- **9-10**

Poster Abstracts -- **11-23**

GEMS Sponsors -- **24-28**

GEMS WEBSITE (URL) and Notes--- **29-30**

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PRESIDENT'S MESSAGE

Welcome to the 34th Annual GEMS Fall Meeting! Brian Chorley, Ph.D., President-Elect and Program Chair, has built an exceptional program around the theme of "Mitochondrial DNA Mutagenesis and Human Health Impacts." For the Spring Meeting, Brian proposed and took on the monumental task of coordinating the first GEMS meeting to feature the ever increasing importance of science communication and outreach. Among many notable features of this meeting, Nobel laureate Oliver Smithies, Ph.D., gave an enthralling and inspirational talk on his scientific journey, attendees heard encouraging messages from several distinguished speakers to share their science with the public, and it was also the first time that high school students interested in pursuing scientific careers were invited to attend a GEMS meeting. Brian has also been dedicated to other aspects of strengthening the Society. GEMS is truly fortuitous to have had such a committed, enthusiastic, and energetic person to serve as President-Elect, and there is no doubt in my mind that Brian will be a legendary GEMS President.

It has been an honor and a privilege to serve as the President of GEMS. During my tenure as President-Elect for GEMS, I had also served as a Councilor appointed to the Executive Board of the Environmental Mutagenesis and Genomics Society (EMGS). During that time, I learned a lot about the nuts and bolts that go into running a society. My goal as President was to apply what I had learned by promoting the implementation of an integrated membership management software system to streamline GEMS operations. Among other advantages, GEMS now has an information-rich website that includes a secure, online payment system for membership dues and meeting registration fees, with invoices for every transaction. It has never been easier to join GEMS and maintain membership, and members have the option of creating a profile. GEMS has only begun to tap into the potential of this system. I'm excited to see how it will be used by future Boards to boost networking and career development opportunities for area scientists, to enhance the community within GEMS, and to promote interaction between GEMS and other scientific organizations in the RTP. GEMS is a non-profit organization and we are indebted to our sponsors for their support of the Fall Meeting. We have benefited from the generous financial assistance of the NIEHS, the Burroughs Wellcome Fund, and many corporate sponsors. Please visit with company representatives during the meeting; they are here today to equip us with materials that can help us to do our work with greater speed and success!

GEMS was founded in the spirit of providing early career scientists and staff scientists with opportunities to showcase their work. Thank you to the talented and dedicated trainees and technicians who have shared their work today with the GEMS community. GEMS offers the opportunity to compete for the Best Talk Award, sponsored by a generous contribution from Mr. Tom Hughes, GEMS Past President. The awardee will also receive the EMGS Emerging Scientist Award. These awards provide support for the recipient to attend the 2017 EMGS Annual Meeting, which will be held in Raleigh. Poster presenters will compete for First, Second, and Third prizes, kindly sponsored by the Burroughs Wellcome Fund, which is committed to enhancing the professional development of early career scientists. In addition to providing a venue for early career scientists to shine and to hone their networking skills, GEMS celebrates the accomplishments and service of scientists who have advanced further in their careers with the Lifetime Achievement Award. There are many deserving candidates for this award, and this year, we had some catching up to do! Please congratulate Jack Bishop, Ph.D., John (Jef) French, Ph.D., RoseAnne McGee, B.S., and Kristine Witt, M.S. for their outstanding scientific contributions and for their exemplary dedication to GEMS. Please see the GEMS website to learn more about the award and to read inspiring interviews given by the Lifetime Achievement awardees.

Running GEMS and putting on two meetings a year is a team effort! I have greatly enjoyed working with a truly delightful and dedicated Board of Directors. Please join me in thanking Holly Mortensen (Secretary), Jef French (Treasurer), Brian Chorley (President-Elect) and Councilors Michelle Campbell, Jenna Currier, Nagu Keshava, Janice Lee, Nancy Hanley, Erin Hines, Jennifer Nichols, Caren Weinhouse, and George Woodall for their many contributions. Carol Swartz, our Corporate Sponsor Coordinator, Kristine Witt, who has coordinated procuring our GEMS awards and mementos, and Carolyn Harris, who has for many years maintained our membership records, also deserve our recognition and thanks for their constant and much appreciated efforts.

Please welcome our new Board of Directors members, including Holly Mortensen (President-Elect), Nisha Sipes (Secretary), Lisa Smeester (Treasurer), and Councilors Tom Hughes, Natalia Ryan (née Van Duyn), and Catherine Sprankle. I and many others have found our involvement with GEMS to be a greatly rewarding experience and I strongly encourage members to get involved by running for office. Last but not least, another way to get involved is to serve as a judge for the poster and platform competitions, and I would like to thank the volunteers who make the competitions possible!

GEMS is a truly unique and vibrant society, nurtured by the outstanding scientific talent here in the RTP and surrounding areas. The continued success of the Society depends on the active support of members. Thank you for your support and enjoy the meeting!

Stephanie L. Smith-Roe, Ph.D.
GEMS President, 2015 - 2016

AGENDA

Mitochondrial DNA Mutagenesis and Human Health Impacts

8:00 - 8:45 a.m. **Registration and Continental Breakfast**

8:45 – 9:00 a.m. **Welcome**
Stephanie Smith-Roe, Ph.D., President
Brian Chorley, Ph.D., President-Elect

9:00 – 9:45 a.m. **The Role of DNA Polymerase Gamma in Mitochondrial DNA Mutagenesis**
William C. Copeland, Ph.D., NIEHS, RTP, NC

9:45 – 10:30 a.m. **Therapeutics for Mitochondrial DNA Instability**
Sherine S.L. Chan, Ph.D., Medical University of South Carolina, Charleston, SC

10:30 – 12:00 p.m. **Poster Session and Sponsor Exhibits**
10:30 – 11:15: Odd posters attended
11:15 – 12:00: Even posters attended

12:00 – 1:15 p.m. **Lunch (provided with registration)**
Lifetime Achievement Awards
Business Meeting

1:15 – 2:00 p.m. **Mitochondria as a Target of Environmental Toxicants**
Joel N. Meyer, Ph.D., Duke University, Durham, NC

2:00 – 2:45 p.m. **Trainee Platform Presentations**
2:00 – 2:15: **Axel J. Berky**, Duke University, Durham, NC
2:15 – 2:30: **Kirsten C. Verhein**, NIEHS, RTP, NC
2:30 – 2:45: **Colette N. Miller,** US EPA, RTP, NC

2:45 – 3:15 p.m. **Break (beverages and snacks)**

3:15 – 4:00 p.m. **Mitochondrial Regulation of the Epigenome and Transcriptome**
Janine H. Santos, Ph.D., NIEHS, RTP, NC

4:00 – 4:30 p.m. **Awards and Closing Remarks; Adjourn at 4:30 p.m.**

MEET OUR INVITED SPEAKERS

William C. Copeland, Ph.D.
Chief, Genome Integrity and Structural Biology Laboratory and Principal Investigator, Mitochondrial DNA Replication Group, Genomic Integrity and Structural Biology Laboratory, National Institute of Environmental Health Sciences, NIH,
Research Triangle Park, North Carolina, 27709

I received my Ph.D. in chemistry/biochemistry from the University of Texas at Austin in 1988 under the supervision of Dr. Jon Robertus studying histidine decarboxylase. I then completed my postdoctoral training at Stanford University School of Medicine, Department of Pathology with Dr. Teresa Wang, studying the human DNA polymerase alpha/primase complex. In 1993, I joined the National Institutes of Environmental Health Sciences, NIH, in Research Triangle Park, NC and am currently the head of the Mitochondrial DNA Replication Group in the Genome Integrity and Structural Biology Laboratory within the Intramural Research Division. I am also the Chief of the Genome Integrity and Structural Biology department at the NIEHS.

The primary goal of the Mitochondrial DNA Replication Group is to understand the role of the replication apparatus in the production and prevention of mutations in mtDNA. Because the genetic stability of mtDNA depends on the accuracy of DNA polymerase gamma (pol γ), this project focuses on understanding the role of human pol γ in mtDNA mutagenesis. Furthermore, nearly 300 disease mutations in the POLG gene for the catalytic subunit of pol γ have been linked to several mitochondrial disorders, including progressive external ophthalmoplegia, sensory and ataxic neuropathy, Alpers syndrome, and male infertility. We are studying the molecular effects of disease mutations in pol γ, its accessory subunit and the mitochondrial DNA helicase. The current projects address the role of human pol γ in mtDNA mutagenesis; study of the molecular effects of disease mutations in pol γ; and are elucidating the role of the human pol γ in induced mitochondrial toxicity caused by anti-HIV nucleoside analogs. My group has over 20 years of experience in research of mitochondrial DNA replication and this group has pioneered the characterization of the human DNA polymerase complex.

Sherine S. L. Chan, Ph.D.
Associate Professor,
Drug Discovery and Biomedical Sciences,
College of Pharmacy, Medical University of South Carolina,
Charleston, SC 29425

I have been in the mitochondrial field since 1996. I first worked with Dr. Jim Cummins at Murdoch University, Australia, investigating the roles of mitochondrial DNA (mtDNA) mutagenesis in male infertility. Later, I joined Dr. Bill Copeland's laboratory at NIEHS/NIH in Research Triangle Park, NC, where I focused on mtDNA-related mitochondrial diseases using in vitro biochemistry, studies of mitochondrial disease patient cells, and in vivo mouse models of mitochondrial dysfunction. I started my lab at the Medical University of South Carolina in 2009, where we have developed new zebrafish models of mitochondrial dysfunction that recapitulate human disease. These models are now used for studying disease progression, drug discovery and for toxicology studies. We have developed many new assays for studying mitochondrial dysfunction in vivo in the zebrafish. We are focused on understanding certain tissues are more susceptible to mtDNA instability, and how common environmental exposures modulate disease outcome. The long-term goal of my laboratory at the Medical University of South Carolina is to manipulate the mechanisms required to maintain mitochondrial homeostasis to prevent or treat mitochondrial dysfunction. I am also the Co-Founder of Neuroene Therapeutics, a startup drug discovery company based on new non-toxic compounds that improve mitochondrial health.

Joel N. Meyer, Ph.D.
Associate Professor, Nicholas School of the Environment,
Integrated Toxicology and Environmental Health Program, Duke Cancer Institute
Duke University
Durham, NC 27708

Dr. Meyer received a B.S. from Juniata College in 1992 (Environmental Studies, Peace and Conflict Studies), and then moved to Guatemala where he worked in a number of fields including appropriate technology and high school teaching. Working with cookstoves and water pollution led to an interest in environmental health, and he earned a Ph.D. in Environmental Toxicology from Duke University in 2003. This led to an interest in toxic effects on mitochondria and DNA and postdoctoral research with Dr. Bennett Van Houten at NIEHS (2003 to 2006). Dr. Meyer joined the Nicholas School of the Environment at Duke University in 2007. He is currently an associate professor at the Nicholas School of the Environment, faculty member of the Integrated Toxicology and Environmental Health Program and the Duke Cancer Institute, and has a secondary appointment in Duke Civil and Environmental Engineering. His group studies the effects of pollutants on health, with a particular focus on mitochondria and DNA and how the effects of pollutant exposures are different when exposures occur early in life, or in the context of genetic differences. His group studies these effects using the nematode model organism *Caenorhabditis elegans*, cell culture, and collaboratively in samples from people, fish, and other species.

Janine H. Santos, Ph.D.
Mammalian Genome Group, Genomic Integrity and Structural Biology
National Institute of Environmental Health Sciences, NIH
Research Triangle Park, North Carolina, 27709

Janine H. Santos received her Ph.D. in Genetics and Molecular Biology from the Federal University of Rio Grande do Sul in Porto Alegre, Brazil. During her Ph.D. studies. Dr. Santos worked on Genetic Toxicology using *Drosophila melanogaster* as a model system to understand the effects of dietary compounds under conditions of genotoxic stress. She then moved to NC for her post-doctoral fellowship at NIEHS to work on mitochondrial DNA metabolism. In 2006, Dr. Santos became faculty at the New Jersey Medical School and in 2013 returned to NIEHS. Her current line of research involves defining the impact of mitochondrial metabolism to the epigenome and transcriptome in order to better understand the effects of environmental agents that target this organelle.

ABSTRACTS

Invited Talks

Gene-Environment Interactions and mitochondrial DNA mutagenesis in POLG Diseases

William C Copeland, Ph.D.

Mitochondrial DNA Replication Group, Genomic Integrity and Structural Biology Laboratory, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, North Carolina, 27709.

Mitochondrial DNA (mtDNA) is replicated by DNA polymerase gamma which is composed of three subunits encoded by two genes: *POLG* encoding the catalytic subunit (p140) and *POLG2* encoding the homodimeric accessory subunit (p55). Mutations in *POLG* are one of the most common causes of mitochondrial disorders and give rise to mtDNA depletion, deletions and point mutations. To date, there are nearly 300 disease mutations in *POLG* that are linked to mitochondrial diseases such as myocerebrohepatopathy, Alpers-Huttenlocher syndrome, myoclonic epilepsy myopathy sensory ataxia, ataxia neuropathy spectrum and progressive external ophthalmoplegia (PEO). Mutations in *POLG2* and the Twinkle helicase have also been shown to cause PEO and similar disorders. Using biochemical analysis, structural modeling, yeast genetics and mouse models, we are determining the consequences of disease mutations in *POLG, POLG2*, and the Twinkle helicase. Whereas documenting the biochemical defects of *POLG*, *POLG2*, and Twinkle disease variants *in vitro* can be straightforward, predicting the age of phenotypic onset for *POLG* related diseases can be more challenging. Indeed, large differences in age of onset can occur in people bearing the *same POLG* mutations, indicating the existence of confounding factors beyond more obvious biochemical defects. To better address these discrepancies, we are investigating environmental modulators of disease variants, and we have identified unique gene-environment interactions with certain *POLG* disease mutations that sensitize mtDNA to enhanced mutagenesis. We have also developed model systems with cultured cells to monitor the sub-cellular localization of disease variant proteins and their effects on cellular bioenergetics.

Therapeutics for Mitochondrial DNA Instability

Sherine S. L. Chan, Ph.D.

Drug Discovery and Biomedical Sciences, College of Pharmacy, Medical University of South Carolina, Charleston, SC 29425.

Mitochondrial dysfunction is an important contributor to many common human diseases. A key component that is often affected and leads to disease states is mitochondrial DNA (mtDNA) stability. A recent study showed that 90% of currently healthy humans harbor at least one mutation in their mtDNA, and 20% harbor a pathogenic mtDNA mutation. Normal mtDNA replication is an important source of mutation, and more than 2% of the population carries a pathogenic mutation in nuclear-encoded mtDNA maintenance genes. Furthermore, we have a poor understanding of why certain tissues are more affected than others, and there is a lack of relevant animal models that can be used both for understanding disease pathogenesis and progression, as well as for toxicology and drug discovery. Because of these issues, we still do not have effective treatments for mtDNA instability. My lab has developed and validated new and unique zebrafish models of mtDNA instability that we are now using for drug discovery. These genetic models show that severe depletion of mtDNA and the presence of deletions precede alterations in energetics, and that there are tissue-specific differences for both parameters that recapitulate the human condition. We are screening compounds that improve mtDNA stability in vivo, and are developing active compounds as novel therapies for mitochondrial disease.

Roles of mitochondrial fusion, fission, and autophagy in response to environmental mitotoxicants

Joel N Meyer, Ph.D.

Nicholas School of the Environment, faculty member of the Integrated Toxicology & Environmental Health Program and the Duke Cancer Institute, Duke University, Durham, NC 27708-0328.

Many mitochondrial homeostasis genes are human disease genes, and deficiencies in these genes may sensitize to exposures. Mitochondrial DNA (mtDNA) lacks some repair pathways that are present in the nucleus, such that damage formed after exposure to important genotoxins including ultraviolet C (UVC) radiation and some polycyclic aromatic hydrocarbons is not repaired in mtDNA. We found that while persistent, such irreparable damage is slowly removed in a process dependent at least in part on mitochondrial fusion, fission, and autophagy. Furthermore, deficiencies in these processes sensitive the model organisms *Caenorhabditis elegans* to exposures that generate irreparable mtDNA damage. This may have implications for human health, because these processes are dependent on proteins encoded by human disease genes, and are also modulated by lifestyle factors such as diet and exercise. Mutations in mitochondrial fusion, fission and autophagy genes exacerbate the toxic effects of other environmental and pharmaceutical agents, not all of which are genotoxic, including arsenic, cisplatin, rotenone, and paraquat. Others have reported that inhibition of mitochondrial dynamics affects transmission of mtDNA mutations. Finally, we have found that in some cases, developmental exposures to mitochondrial toxicants can result in lifelong and heritable (to offspring) alterations in mitochondrial function. Metabolomic data suggest that this occurs as a result of persistent diversion of metabolic intermediates towards protection against mitochondrial oxidative stress, a model that we are currently testing. Overall, our results suggest a potent gene-environment interaction in which the effects of mtDNA damage are exacerbated by decreased mitochondrial homeostasis.

Mitochondrial regulation of the epigenome and transcriptome

Janine H. Santos, Ph.D.

Genome Integrity and Structural Biology, Biostatistics and Bioinformatics Branch, National Institute of Environmental Health Sciences, National Institutes of Health, Durham, NC 27709.

Mitochondria are well-recognized for their role in ATP and reactive oxygen species generation, in addition to producing intermediate metabolites through the TCA (tricarboxylic acid) cycle. The crosstalk between metabolism and epigenetic modifications in the nucleus is becoming increasingly evident but the extent to which mitochondrial metabolites are required for these effects remains largely unknown. We recently showed using a cell culture model of acute mitochondrial DNA (mtDNA) depletion that electron flow sustains a functional oxidative TCA cycle, which in turn is necessary to maintain histone acetylation in the nucleus. Following this work, we have interrogated the degree of gene expression changes associated with modulation in histone acetylation and in DNA methylation driven by mitochondrial dysfunction. Results will be discussed in light of our recent findings.

ABSTRACTS

Short Talks

T1

Epidemiological Survey on Mitochondrial DNA Copy Number and Mitochondrial DNA Damage in the Peruvian Amazon

Axel J. Berky[2], Ian T. Ryde[2], Ernesto J. Ortiz[1], Beth J. Feingold[3], Heileen Hsu-Kim[2,4], Joel N. Meyer[2], William K. Pan[1,2]
[1]Duke Global Health Institute, Duke University, Durham, NC 27710; [2]Nicholas School of the Environment, Duke University, Durham, NC 27708; [3]University of Albany, School of Public Health, Albany, NY 12114; [4]Duke Pratt School of Engineering, Duke University, Durham, NC 27708

The purpose of the study is to determine the impact of Hg exposure, nutrition and health behaviors on mitochondrial DNA copy number (mtDNA CN) and mitochondrial DNA (mtDNA) damage in peripheral white blood cells (n = 85 participants) in indigenous and non-indigenous communities in the Peruvian Amazon. Nine communities along the Madre de Dios River in Southeastern Peru were sampled in 2014 for mercury in hair, hemoglobin, anthropometry, mtDNA damage and mtDNA CN. Three communities are rural and located in pristine jungle in the river's headwaters upriver from artisanal gold mining (the putative source of mercury exposure), three are urban communities located downriver from artisanal gold mining, while the last three are rural communities located far downriver near the Peruvian border with Bolivia. Households in each community were selected randomly and invited to participate in the study until four houses were enrolled. mtDNA lesions were measured by quantitative long-range PCR, which measures any damage that inhibits the DNA polymerase used in the assay. Copy number was measured using real-time PCR. mtDNA CN was not associated with total Hg exposure in hair, but was positively associated with vegetable consumption ($p<0.06$). mtDNA damage was significantly associated with mercury exposure when considering fruit consumption in random effects models. Being obese led to 0.2 fewer lesion/10kb ($p<0.018$). To our knowledge, this is the first study done in the Peruvian Amazon in which indigenous and non-indigenous communities have well-quantified mercury exposure and detailed information for other mitochondrial health risk factors.

T2

Inter-Strain Variation in Mouse Mitochondrial Genome and Effects of Oxidative Stress

Kirsten C. Verhein[1], Adam Burkholder[2], Jennifer Nichols[1], Zachary McCaw[1], Jacqui Marzec[1], Wesley Gladwell[1], Nicole Reeves[3], Jason Malphurs[3], Greg Solomon[3], Tim Wiltshire[4], David Fargo[2], Bennett Van Houten[5], Steven R. Kleeberger[1]
[1]Immunity, Inflammation & Disease Laboratory, [2]Integrative Bioinformatics Support Group, [3]Epigenetics & Stem Cell Laboratory, National Institute of Environmental Health Sciences, RTP, NC 27709; [4]Eschelman School of Pharmacy, UNC Chapel Hill, NC 27599; [5]Department of Chemical Biology, University of Pittsburgh, Pittsburgh, PA 15261.

Reactive oxygen species contribute to the pathogenesis of many acute and chronic pulmonary disorders, including bronchopulmonary dysplasia (BPD), a respiratory condition affecting preterm infants. BPD treatment often involves respiratory support with high oxygen, and oxidative stress is an adverse effect associated with impaired lung development and function in a subset of infants. Genetic polymorphisms in a few candidate genes have been

associated with BPD susceptibility, however the genetic basis of differential susceptibility remains poorly understood. To mimic features of BPD we developed a mouse model where one day after birth neonatal mice from 29 inbred strains were exposed for 72 hr to normoxia (room air, 20% O_2) or hyperoxia (>95% O_2). Hyperoxia induced phenotypes similar to BPD when compared to normal postnatal lung development in normoxia. We ultra-deep sequenced neonatal lung mitochondrial DNA from these mice and found three major haplotype groups among 27 strains that were sequenced, with classical inbred strains in haplotype 1, NZB and NZO in haplotype 2, and PWD/PhJ and PWK/PhJ in haplotype 3. Wild derived strains had more variation than classical inbred strains. We found exposure to low (20%) and high (>95%) concentrations of oxygen early in life cause lesions in mouse lung mtDNA and nucDNA that are strain dependent. Ultra-deep sequencing also identified mtDNA sequence variation and differences in heteroplasmy and indels across inbred mouse strains that associate with disease phenotypes. Through these combined approaches, we have identified novel candidate susceptibility genes that may improve our understanding of neonatal lung injury and development.

T3
Sex Differences in Placental Mitochondrial Function Associated with Ozone-Induced Fetal Growth Restriction.

Colette N. Miller[1], Katelyn S. Lavrich[2], Danielle Freeborn[3], Janice A. Dye[1], Prasada R. Kodavanti[3], Urmila P. Kodavanti[1]
[1]EPHD, [3]TAD, NHEERL, US EPA, Research Triangle Park, NC 27711; [2]CIT, UNC Chapel Hill, Chapel Hill, NC 27599

Fetal growth restriction is a major underlying cause of infant mortality worldwide. Unfortunately little is known about the mechanisms that drive compromised growth and the role of placental maladaptation on fetal development. In the current study placentas from male and female rat pups were harvested on gestational day (GD) 21 from Long Evans dams exposed to filtered air or 0.8 ppm ozone, 4hr/day during the implantation period on GD 5 and 6. At GD21, pups from ozone exposed dams had reduced weight compared to air control pups. Bioenergetics, measured as oxygen consumption rate (OCR), on mitochondria were measured using the Seahorse XF96 analyzer. Baseline OCR in the female placentas were elevated compared to males. While maternal ozone exposure did not impact female placental OCR at baseline, placentas of ozone-exposed males had increased OCR relative to controls. Following stimulation of the electron transport chain with ADP, a near significant effect of ozone to increase OCR was observed. Gene expression experiments confirmed elevated mitochondrial biogenesis in ozone-exposed male and female placentas. Further, female placentas from ozone-exposed dams had reduced *Sod1* and increased *Bcl2* expression compared to air-exposed female placentas. This difference was not observed in males. Together our data supports the hypothesis that placental mitochondrial dysfunction is related to reduced fetal weight. We demonstrate clear sex differences in the placental mitochondria of both healthy and growth compromised pups. Our findings support the emerging importance of mitochondrial function in the etiology of fetal growth restriction. This abstract does not reflect US EPA policy.

ABSTRACTS

Posters

P1

Incorporation of Human Relevant Metabolism for the Classification of Xenobiotics as Mutagens

Pergentino Balbuena[1], Katherine Dunnick[1], Martin Phillips[1], Jeffrey Enders[1], Susan Ross[1], David Billings[1], Rebecca Clewell[1], Miyoung Yoon[1]

[1]ScitoVation, LLC, Research Triangle Park, NC 27709

Currently, induced rat S9 fraction is used to address metabolic activation in *in vitro* genotoxicity testing. However, this approach lacks human relevance and also is one of the major reasons for high false positive rates in *in vitro*-based screening for genotoxicity. We have developed a method using long-term primary human hepatocyte cell cultures to generate a mixture of human-relevant metabolites that can be tested for genotoxicity *in vitro*. Two case compounds, 1,7-octadiene (OCTA) and cyclophosphamide (CP), were tested for genotoxicity using the *in vitro* the micronucleus (MN) assay with and without metabolic competence to show the limitation of the current rat S9-based approach. Both compounds require cytochrome P450-mediated bioactivation to elicit genotoxicity. However, *in vivo*, their active metabolites are detoxified by various enzymatic and non-enzymatic pathways, some of which will not be captured by adding induced rat S9 to *in vitro* toxicity assays. We tested the genotoxic potential of OCTA and CP, along with their bioactivated metabolites, 1,2:7,8-diepoxyoctane (DEO) and 4-hydroxycyclophosphamide (hydroxyCP) the MN assay in human fibrosarcoma cells. As expected, the parent compounds were inactive, while their metabolites were genotoxic (EC_{50} at 11.12 μM DEO and 1.53 μM hydroxyCP). Our integrated *in vitro* genotoxicity testing method will support *in vitro*-based evaluation of concentration-response for genotoxicity under human, *in vivo*-relevant exposure conditions and will thereby increase confidence in *in vitro*-based prediction of human safety exposure to potential genotoxic compounds.

P2

Sexual epigenetic dimorphism in the human placenta: Implications for susceptibility to stressors during the prenatal period

Elizabeth Martin[1], Lisa Smeester[1], Paige A. Bommorito[1], Matthew R. Grace[2], Kim Boggess[2], Karl K. Kuban[3], T. Michael O'Shea[4], Rebecca C. Fry[1,5]

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The placenta is a temporary organ responsible for providing oxygen and nutrients to the fetus. It also serves as the primary membrane through which environmental contaminants pass and accumulate. Thus it is an important organ for understanding responses to the prenatal environment. It has been observed that many later life health outcomes associated with prenatal exposures are sexually dimorphic. Similarly, it has been noted that there are physiological differences between placentas of male and female infants. Still, the factors that establish these differences are not known. DNA methylation could play an important role in determining differences in the

placenta. In this study, we investigated the differences in DNA methylation using the Illumina 450k Human Beadchip between placentas from male and female pregnancies in the Extremely Low Gestational Age Newborns (ELGANs) cohort and replicated these findings in a separate US-based cohort. We identified n=4,371 differentially methylated CpG sites ($p<1x10^{-7}$) between male and female placentas in the ELGANs cohort, representing 388 genes. We validated n=2,745 of these sites in a separate US-based cohort (n=587 genes). Among the validated set were genes that play key roles in immune functions (n=31), transport (n=119), and growth/transcription factors (n=6). We also identified enrichment of transcription factor binding sites for NFATC, and PAX4 among hypermethylated genes, MAZ, and FOXO4 among hypomethylated genes. SP1 was found to be enriched in both hyper- and hypo-methylated gene sets. These data suggest a strong epigenetic patterning related to sex of the infant and could provide insight into sex-based risk of disease.

P3

Differential DNA Methylation in Circulating Monocytes links Cigarette Smoke to Human Disease

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Cigarette smoke influences DNA methylation and human diseases. However, the mechanistic links between smoking-responsive methylation and related diseases remain unknown. Here we investigated genome-wide smoking-associated differential methylated regions (SM-DMRs) by Reduced Representation Bisulfite Sequencing (RRBS) using primary monocytes from two independently recruited subject groups. We found that SM-DMRs preferentially occur at enhancers with characteristics of potential open chromatin and transcription factor (TF) binding indicated by ENCODE data, suggesting the crosstalk between DNA methylation, TF binding, chromatin state of enhancer, and cigarette smoking exposure. DMRs often associate with the differential expression of their nearby genes and corresponding noncoding or enhancer RNAs. Our recent study revealed that the methylation of cg05575921 CpG site, within Aryl-Hydrocarbon Receptor Repressor (*AHRR*) SM-DMR, by 450K array significantly mediated the association between smoking exposure and subclinical atherosclerosis. The *AHRR* SM-DMR represents the most significant DMR in the present study. We further validated *AHRR* SM-DMR by BiSulfite Amplicon Sequencing (BSAS) in monocytes and a novel CpG site was determined to be more sensitive to smoking exposure than the most significant CpG (cg05575921) previously identified by 450K array. These findings were recapitulated in saliva by BSAS, suggesting saliva may provide an alternative resource for evaluating smoking exposure and related atherosclerosis. Mechanistically, our results indicated *AHRR* SM-DMR regulates *AHRR* mRNA expression through activating *AHRR* enhancer activity indicated in monocytes but not in granulocytes. This study suggests a mechanism by which SM-DMR regulates target gene expression through modulating enhancer activity and may contribute to smoking-related disease in cell-type specific manner.

P4

An Embryonic Zebrafish Screening Method for Early Life-Stage Obesity

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The chemical environment in the womb may increase susceptibility to different life-stage and life-long metabolic diseases including obesity. Specifically, evidence suggests fetal obesogen exposure may shift multipotent stromal stem cell differentiation disproportionately toward the adipocyte pool. This chemical disruption of adipocyte organogenesis may increase the number and size of fat depots later in life, rendering offspring more susceptible to obesity. The challenge is to understand if environmental chemical exposures during developmentally sensitive windows affect adipose mass later in life. *In vitro* models lack the integrated systems approach needed to assess adipose development, while mammalian models are impractical in a screen of thousands of chemicals. Therefore, an optimal zebrafish obesogen screening method was developed to examine the effect of embryonic chemical exposure on life-stage adipose mass. A time-line for adipose depot formation was mapped in zebrafish 6−14 days post fertilization (dpf) using the lipophilic dye, Nile Red, in combination with fluorescent microscopy and gene expression. Those time points were then used to evaluate embryonic tributyltin chloride (TBT, a known obesogen) exposure on adipose mass. Embryos exposed to TBT (10nM daily renewal, 0−5 dpf) had increased adipogenic and early commitment markers *PPARγ*, *Cidec*, and *Znf423* (1.8-, 1.6 and 3.2-fold, respectively; $p < 0.05$), and developed adipose depots that were larger (3.7-fold at 10 dpf, $p = 0.0255$) and appeared 2 days earlier (8 dpf versus 10 dpf) than controls. These results suggest the zebrafish model as a promising new tool to screen for chemical obesogens. *This abstract may not necessarily reflect official Agency policy.*

P5

Synergistic Effects of the *in cis* T251I and P587L DNA Polymerase γ (PolG) Disease Mutation

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Human mitochondrial DNA (mtDNA) polymerase gamma (Pol γ) is the only polymerase known to replicate and maintain the genetic integrity of the mitochondrial genome. Mutation of *POLG* impedes maintenance of mtDNA and causes mitochondrial disease. Two common *POLG* mutations found in patients with mitochondrial disease generate T251I and P587L amino acid substitutions in Pol γ. Rarely reported independently, the T251I and P587L mutations are usually found *in cis*. To understand whether T251I or P587L is the primary pathogenic allele or whether both substitutions are required to cause disease, we overexpressed and purified WT, T251I, P587L and T251I + P587L double variant forms of recombinant Pol γ. Biochemical characterization of the variants revealed impaired DNA binding affinity, reduced thermostability, diminished exonuclease activity, defective catalytic activity and compromised DNA processivity even in the presence of the accessory subunit that functions to promote processivity. However, physical association with the accessory subunit was unperturbed, which indicates inter-subunit affinities similar to WT. Notably, although the single mutations were similarly

impaired, a synergistic effect was found for the double mutation across all parameters. In conclusion, our biochemical analyses suggest individual T251I and P587L substitutions functionally impair Pol γ, with greater pathogenicity predicted for the single P587L variant. Combining T251I and P587L induces extreme thermal lability and leads to synergistic nucleotide and DNA binding defects, which severely impair catalytic activity and correlate with presentation of disease in patients.

P6

Transcriptome Profiling in Hippocampal Dendrites Reveals a Role for Mitochondria in CA2 Physiology and Function

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Several lines of evidence suggest that experience-dependent translation of mRNA in neuronal dendrites is required for the persistence of experience-dependent changes in the brain. This spatial restriction of mRNA to subcellular domains enables local regulation of protein synthesis at synaptic sites. Synapses in hippocampal area CA2 differ from neighboring subregions in that they do not typically strengthen, a process that requires local protein synthesis. In fact, we found that even the maintenance of baseline synaptic transmission in CA2 may require protein synthesis, as translation inhibitors led to a decrease in postsynaptic responses. These data suggest that local protein synthesis may play a critical role in gating synaptic plasticity in CA2 dendrites. To identify the RNA transcripts in CA2 and surrounding subregion dendrites (CA1, CA3 and DG), we used laser-capture microdissection and RNA-Seq. We found that CA2 cell bodies and dendrites are enriched for unique mRNA transcripts compared to neighboring subregions. In particular, plasticity-restricting mRNAs, such as *Pcp4, Rgs14, Ptpn5* and *Necab2,* were enriched in CA2 dendrites and validated using single molecule fluorescent in situ hybridization. Pathway analyses of enriched targets in CA2 identified genes critical for mitochondrial function and energy generation. Ongoing studies are testing whether differences in mitochondrial efficiency may be linked to the unique physiology of CA2 neurons, such as their resistance to neuronal injury. Identifying mechanisms underlying neuronal resistance to damage has far-reaching implications for neurological diseases and exposure research where selective populations of neurons are sensitive to environmental toxicants.

P7

Identifying threshold shaped DNA repair processes and the genotoxic tipping point during the p53-mediated DNA damage response

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One of the more contentious conventions in risk assessment is the default assumption of low-dose linearity for genotoxicity. In apparent conflict with this assumption, some genotoxic chemicals exhibit threshold-like responses in mutation and genotoxicity assays. Based on previous work from our group, we hypothesize that at low doses of DNA damage (i.e. few double strand breaks), p53, the DNA repair protein, acts as a cofactor for DNA repair center (DRC) formation promoting efficient DNA repair and damage resolution, while at higher

chemical doses, increased DNA damage depletes these DRC proteins and promotes transition towards transcriptional repair events leading to mutations such as micronucleus formation. This genotoxic “tipping point” between low dose p53-mediated translational repair and high dose (cell damaging) transcriptional repair can give critical insights into assigning risk assessment criteria to various chemicals of human exposure relevance. This study focuses on identifying the genotoxic tipping point of selected genotoxic chemicals by assessing the intersection of saturated DRC formation (pH2AX and p53BP1 foci staining) and late stage cellular adverse effects (micronucleus formation) following chemical exposure in human fibrosarcoma HT1080 cells. Data indicates that a dose-dependent threshold like increase in the magnitude of these two endpoints occurred in similar fashions following treatment with 5 different genotoxic chemicals of varying DNA-damage inducing mechanisms in our model. Preliminary results thus show that assessment of these p53-mediated repair processes for various chemicals may support a better understanding of the threshold shaped mechanism of DNA damage repair and which doses promote “tipping-points” toward cellular death.

P8

Detection of Smoking-Associated DNA Methylation Changes in the AHRR Gene Using High Resolution Melt Method

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Cigarette smoking has been shown to be associated with DNA methylation changes and a variety of smoking-related diseases, including lung cancer, chronic obstructive pulmonary disease, heart disease, stroke etc. DNA methylation at site cg05575921 in the aryl hydrocarbon receptor repressor (*AHRR*) gene has been identified as the most significant smoking-responsive methylation site in many cohort studies using Illumina 450K methylation arrays. Importantly, it has recently been linked to pre-clinical atherosclerosis. Therefore, methylation of this site potentially serves as a biomarker for smoking exposure and smoking-related disease. Compared to other locus-specific DNA methylation approaches, such as pyrosequencing and amplicon sequencing, the high resolution melt (HRM) method is one of the fastest and most cost-effective methods for DNA methylation measurement. Here, we determined smoking-associated methylation changes at site cg05575921 by HRM using genomic DNA of adult whole blood cells from 38 smokers and 45 nonsmokers. We further compared methylation values from HRM and 450K array. We found that DNA methylation at cg05575921 was significantly increased (average methylation difference = 33.23%; P value=6.66071x 10^{-24}) in smokers from our HRM experiments, which is consistent with 450K data. Furthermore, methylation values of cg05575921 from HRM and 450K array were strongly correlated (R^2=0.917692) among the same set of subjects. In addition, the methylation values of cg05575921 were also strongly correlated with cotinine levels, a biomarker of tobacco exposure (R^2=0.9818). Our results suggest that HRM may represent a fast and cost-effective locus-specific method for the validation of DNA methylation changes in response to environmental exposures and their related diseases.

P9
Acute Effects of Mitochondrial-Disrupting Toxicants on Inflammasome Activation

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Inflammasomes are a component of the innate immune system recently shown to contribute to inflammatory disease. They are typically activated by pathogen-associated and damage-associated molecular patterns, involve cleavage of caspase-1 the production and secretion of interleukin-1 beta (IL-1β), and have been associated with mitochondrial damage and reactive oxygen species production. The NTP Tox21 screening efforts have identified several mitochondrial-disrupting compounds. Of these, five organotin compounds, triethyltin bromide (TET Br), trimethyltin hydroxide (TMT OH), bis(tributyltin) oxide, tributyltin chloride, and triphenyltin hydroxide were examined for their potential to initiate a response following lipopolysaccharide (LPS) priming in mouse macrophage RAW 264.7 cells. Cells treated with TET Br (10uM) and TMT OH (1.25uM) for six hours produced a 30-50% disruption in mitochondrial membrane potential (MMP) without overt cell death. Flow-cytometry assays indicated activation of caspase-1 in 20% of the tin-dosed cells. Approximately 20% of cells transfected with a fluorescent biomarker for inflammasome assembly displayed "speck-like" aggregation of the inflammasome complex, consistent with active-caspase-1 presentation. Elevated release of IL-1 cytokine was detected via the HEK-Blue bioassay for IL-1 receptor binding, exhibiting a significant increase over control. Western blot analysis for pro- and mature IL-1β showed production of primarily the pro- form, while the mature form was not detected. Taken together, the data suggests that TET Br and TMT OH, confirmed MMP disruptors, could trigger inflammasome activation in a sub-population of the cells. The release of biologically active IL-1 likely reflects induction of a concurrent non-canonical pathway involving production and secretion of IL-1α.

P10
Rodent Liver Tumor Responses in NTP Studies

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Liver tumor responses are the most common outcomes in the NTP bioassays in rats and mice. In a recent survey of 490 NTP bioassays where the same chemical was tested in both rats and mice, 146 bioassays had a liver tumor response in rats (14), mice (95), or both species (37). The historical control data for liver tumor incidence is 2% and 1% in male and female F344/N rats and 72%, and 35% in male and female B6C3F1 mice, respectively. It is commonly assumed that mice with a high background liver tumor incidence also have a high liver tumor incidence following chemical exposure. Based on this assumption, it is expected that male mice will have a higher incidence of chemical-induced liver tumors than female mice. The liver tumors (hepatocellular adenoma, hepatocellular carcinoma, hepatoblastoma combined) with clear and some evidence of carcinogenicity were observed at 28.8% and 28.8% in male and female F344/N rats and 61.6% and 79.5% in male and female B6C3F1 mice, respectively. These data are not in concordance with the above assumption since female mice had a higher incidence of chemical-induced liver tumors than male mice. The liver tumor incidences in rodent bioassays treated with genotoxic (Ames and/or micronucleus assay) and non-genotoxic chemicals were 25% and 75%,

respectively, suggesting that non-genotoxic mechanisms play an important role in rodent liver tumor response. The poster will attempt to address some of mechanisms of rodent hepatocellular carcinogenesis in NTP studies.

P11

Mitochondrial Variation and Risk of Bronchopulmonary Dysplasia (BPD) in Argentinian Infants

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Rationale: BPD causes vascular damage and impaired lung development in premature infants on mechanical ventilation for respiratory distress. Nuclear-coded antioxidant genes and mitochondrial (mt) variants affect function and contribute to low birth weight and BPD. Excess oxygen damages highly susceptible mtDNA, reducing electron transport chain integrity. We identified significant differences (heteroplasmy and mtDNA sequence) in a neonatal mouse model of hyperoxia-induced BPD that associated with lung phentoypes. In this pilot study we used ultra-deep sequencing to identify mtDNA variations of preterm infants with and without BPD. **Methods:** Saliva samples were collected prior to ventilation. DNA was isolated from Argentinian infants and their parents (12 non-BPD trios, 12 BPD trios) and mtDNA amplified by long range PCR. Purified fragments were tagged with Nextera libraries, and sequenced with a NextSeq 500 instrument. **Results:** Two-fold heteroplasmy increase and 3-fold higher incidence of SNP locus heterozygosity were found in BPD infants compared to non-PBD infants. D loop, MT-ND2 and MT-COI mutations varied most in BPD infants. MT-ND4 and MT-ND6 variants predicted to be damaging (PolyPhen) were 2 fold higher in BPD infants versus controls. Interestingly, two D loop variants previously associated with oxidative stress in adults were differentially distributed in BPD infants compared to controls. **Conclusions:** Mutations to complexes I (ND2, ND4 and ND6) and IV (COI) of the electron transport chain were overrepresented in BPD infants. Mitochondrial polymorphisms may contribute to differential susceptibility to BPD in preterm infants.

P12

Deficiencies in Mitochondrial Fission and Fusion Sensitize *C. elegans* to Arsenite-Induced Mitochondrial Dysfunction

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Despite decades of research, the precise mechanism(s) underlying arsenic-induced disease remain poorly understood. However, mitochondria appear to be an important intracellular target of arsenic toxicity. The trivalent arsenical, arsenite, can induce mitochondrial ROS production, inhibit myriad enzymes involved in energy metabolism, and cause metabolic shifts from oxidative phosphorylation to aerobic glycolysis. As 1 in 4,000 individuals suffer from mitochondrial disease, and millions of people worldwide are chronically exposed to arsenic through contaminated drinking water, it is important to understand how this already afflicted population will respond to arsenic exposure. Using the model organism *C. elegans,* we examine an important subset of mitochondrial disease genes. We show that deficiencies in mitochondrial fission (*DRP1*) and fusion (*MFN2*, *OPA1*) genes, which regulate mitochondrial dynamics, energy production, and stress response, sensitize nematodes to arsenite-induced lethality, and developmental and reproductive toxicity. Furthermore, low-dose

arsenite exposure exacerbates mitochondrial dysfunction in fusion-deficient nematodes (reduces ATP, and basal, maximal and spare respiratory capacity), while potentiating mitochondrial function in wild-type nematodes (increases basal and maximal respiration, citrate synthase activity, mtDNA content), and has limited effect on mitochondrial function in fission-deficient nematodes. Additionally, arsenite inhibits pyruvate dehydrogenase and isocitrate dehydrogenase activity in fusion-deficient nematodes, suggesting disruption of pyruvate metabolism and TCA cycle activity underlie the observed mitochondrial deficits. This research demonstrates the importance of mitochondrial dynamics in limiting arsenite toxicity, and suggests individuals suffering from deficiencies in these processes may be susceptible to arsenite exposure. This work was funded by the NIH and NIEHS (R01-ES017540-01A2 & F31ES026859).

P13
Development of Direct Double Strand Break Labeling Assay for Genotoxicity Assessment

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The development of fit-for-purpose *in vitro* toxicity assays to better predict and define DNA damaging chemicals has become necessary to predict adverse outcomes via *in vitro* rather than *in vivo* methods. Current genotoxicity assays rely on indirect measurements of DNA damage through assessment of DNA repair foci or micronuclei formation. While these provide valuable information, they rely on high chemical concentrations and lack sufficient resolution at human-relevant concentrations. We modified a published method (direct *in situ* labeling of double strand breaks (DSBs) for high-throughput compatibility. Effectiveness of this method to detect DNA double strand breaks was tested against known DNA damaging compounds. Initial studies were conducted utilizing aphidicolin, an inhibitor of DNA polymerase α and δ, as a test compound in HT1080 fibrosarcoma cells. Preliminary data indicate the altered method detects DSBs that closely model data from in-house genotoxicity assays (micronucleus and DNA repair centers). Sensitivity studies using prototype chemicals, etoposide (ETP) and methyl methanesulfonate (MMS) showed the direct DSB labeling method (DDL) to be 10 times more sensitive than traditional micronucleus or DNA repair center assays (detected changes in DSBs compared to control cells at 0.001 vs. 0.02 and 0.01µM ETP and 10 vs. 100 and 60µM MMS, respectively). Based on preliminary data, the DDL method provides a novel tool to determine genotoxic potential at human-relevant chemical concentrations compared to traditional *in vitro* models. Future studies are focused on determining accuracy of the assay using positive, false-positive, and negative controls for genotoxicity, and miniaturizing the assay for high-throughput screening.

P14
Characterization of the First Clinical Homozygous PolG2 Mutation, R182W

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Human mitochondrial DNA (mtDNA) polymerase gamma (Polγ) is the only known replicative polymerase of the mitochondrial genome. Polγ consists of two nuclear encoded subunits, the p140 subunit encoded by the *POLG* gene and a smaller p55 homodimeric accessory subunit encoded by the *POLG2* gene. While PolG functions as the catalytic subunit, PolG2 promotes processivity and enhances PolG's DNA binding. Recently, a patient presented with mitochondrial disease symptoms primarily affecting liver and skeletal muscle. Whole exome sequencing revealed a homozygous missense mutation within PolG2, R182W (Varma et al, 2016). The apo structure of PolG2 (PDB: 2G4C) shows R182W to be located at the base of the dimerization domain, which suggests this mutation may affect dimerization. Characterization of purified R182W PolG2 has shown a 15°C drop in thermostability and an important structural change leading to the mutant eluting on an analytical S200 at a molecular weight of 87kDa (half-way between the size of a monomer and dimer). Atomic Force Microscopy suggests that R182W PolG2 is more compact in both volume and height than WT PolG2. This mutation also exhibits a 10-fold weaker DNA binding affinity but does not significantly affect R182W PolG2's ability to interact with and stimulate the catalytic subunit as assessed by steady state kinetics, processivity, exonuclease inhibition or PolG-PolG2 binding *in vitro*. Patient dermal fibroblasts show a clear growth defect but not reduced bioenergetics. However, HEK cells overexpressing R182W PolG2 have significantly reduced bioenergetics that suggests the reduced stability of the protein affects mitochondrial function *ex vivo*.

P15
Computable AOPs Based on Biological Ontologies

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The Adverse Outcome Pathway (AOP) framework connects molecular perturbations with endpoints used for regulatory decision-making by providing a conceptual construct for toxicity mechanisms. Describing AOPs via formal ontologies can enhance the reusability of Key Events (KEs), enable computation on AOP relationships, and facilitate the development of quantitative AOPs (qAOPs) backed by computational models. KEs representing over 150 AOPs from the AOP-Wiki (http://aopwiki.org/) were annotated using 22 publicly available controlled vocabularies and ontologies following a review of 66 ontologies. Individual KEs were allowed to have one or more "event components" consisting of a biological process, object, and action with each term originating from one of the 22 ontologies. The biological context in which the KE occurs is described using cellular, organ, and species ontologies. The ontologies were ranked based on the number of classes or terms used. The Gene Ontology provided the majority of classes describing processes underlying KEs with 516 of 690 total classes. The Chemical Entities of Biological Interest ontology covered the most objects, with 172 of 565 total classes. Ontologies with infrequently used terms, as low as 1-3 classes across all KEs, will be re-evaluated. For 581

distinct KEs tagged, 762 of 1001 event components identified were reused or partially reused among KEs. Complete reuse of event components suggests that KEs should be merged; partial reuse suggests looser associations among KEs and AOPs. AOPs annotated with controlled vocabularies describing endpoints traditionally used for risk assessment can better highlight their potential applications. [This is an abstract or a proposed presentation and does not necessarily reflect EPA policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.]

P16

Dysregulation of Inflammatory Activation in Murine Microglia Following Arsenic Exposure

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As resident macrophages of the CNS microglia mount an immune response to injury or disease, releasing inflammatory factors including: interleukin (IL)-1α, IL-1β, IL-6, and tumor necrosis factor (TNF)α while phagocytosing aberrant material to maintain homeostasis. Inorganic arsenic (iAs) is reported to disrupt the immune response in macrophages. Murine BV-2 microglia cells were exposed 1 μM iAs for 24h to examine their immune response to iAs. mRNA for IL-1β was elevated by iAs with no change in TNF α, IL-1 α, IL-6. Subsequent challenge with lipopolysaccharide [LPS; 100 ng/mL] for 3h showed significantly diminished elevations in IL-1α, IL-1β, and IL-6 mRNA folloing iAs exposure. Challenge with IL-4/IL-13 [10 ng/mL] for 3h showed a diminished elevation in IL-4 and Arg-1 in iAs exposed cells. iAs exposed microglia showed a shift in phagocytic capacity. When examined in mice exposed to iAs [42.5 ppm] via drinking water for 6 weeks, Iba-1+ microglia in the hippocampus showed diminished ramification with no increase in IL-1α, IL-1β, IL-6, and TNFα mRNA levels. At 3h following LPS [100 μg/kg ip], IL-1α, IL-1β, IL-6, and TNFα mRNA levels in the hippocampus were significantly lower in iAs mice. Following 4 daily i.p. injections of LPS [1mg/kg] to induce an anti-inflammatory response, Arg-1 and YM-1 mRNA levels were significantly lower in iAs mice. Taken together, the effects observed *in vitro* and *in vivo* on the pro- and anti-inflammatory response suggest that iAs exposure modifies the ability of the resident brain immune cells to mount an appropriate innate immune response.
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P17

Informatics approaches in the Biological Characterization of Adverse Outcome Pathways

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Adverse Outcome Pathways (AOPs) are a conceptual framework to characterize toxicity pathways by a series of mechanistic steps from a molecular initiating event to population outcomes. This framework helps to direct risk assessment research, for example by aiding in computational prioritization of chemicals, genes, and tissues relevant to an adverse health outcome. We have designed and implemented a computational workflow to access a wealth of public data relating genes, chemicals, diseases, pathways, and species, to provide a biological context for putative AOPs. We selected three AOP case studies: ER/Aromatase Antagonism Leading to Reproductive Dysfunction, AHR1 Activation Leading to Cardiotoxicity, and AChE Inhibition Leading to Acute Mortality, and deduced a taxonomic range of applicability for each AOP. We developed computational tools to automatically access and analyze the pathway activity of AOP-relevant protein orthologs, finding broad similarity among vertebrate species for the ER/Aromatase and AHR1 AOPs, and similarity extending to invertebrate animal species for AChE inhibition. Additionally, we used public gene expression data to find groups of highly co-expressed genes, and compared those groups across organisms. To interpret these findings at a higher level of biological organization, we created the AOPdb, a relational database that mines results from sources including NCBI, KEGG, Reactome, CTD, and OMIM. This multi-source database connects genes, pathways, and chemicals relevant to an AOP, filling annotation gaps faced by methods that rely on data from a single data source. We demonstrate how the AOPdb aids in hypothesis testing through biological characterization of a given AOP using guided queries. *The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.*

P18

Human mitochondrial DNA haplogroup M8a potentially enhances the penetrance of m.8684C>T in non-obstructive azoospermia

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Non-obstructive azoospermia (NOA) is one of the most severe forms of idiopathic male infertility, the pathophysiology of which is largely unknown. Mitochondrial DNA (mtDNA) is believed to be both the source and target of reactive oxygen species (ROS), and its variants could induce meiotic arrest. To explore the role of mtDNA in NOA, a two-stage study was performed in a Han Chinese Population. In the screening stage, the mtDNA genome was sequenced in 92 NOA cases and 95 healthy controls using next generation sequencing

(NGS). Thirteen mtDNA haplogroups (hgs) were ascertained, and ten susceptible variants were observed. In the validation stage, we found that individuals with hg M8* were associated with increased risk of NOA (odds ratio [OR] 2.61, 95% confidence interval [CI] 1.47-4.61) (P=6.76×10^{-4}). Unexpectedly, the frequency of m.8684C>T, the defining marker for hg M8a, was also higher in the case group (OR 4.14, 95% CI 1.56-11.03) (P=2.09×10^{-3}). Subsequently, we compared the frequency distributions of the sub-hgs of hg M8* (including hgs M8a and Z) and, intriguingly, no significance was found in hg Z. Additionally, antioxidant capacity, including total antioxidant capability (T-AOC) and superoxide dismutase (SOD) were evaluated. The level of T-AOC was significantly decreased (P<0.05) compared to the control group. These results demonstrated that hg M8a background in general enhances the penetrance of m.8684C>T in NOA. And mtDNA genetic variants, causing low antioxidant levels, might increase mitochondrial damage and impair normal spermatogenesis.

P19
Evaluation of Air-Liquid Interface Exposure Systems for In Vitro Assessment of Airborne Pollutants

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Exposure of cells to airborne pollutants at the air-liquid interface (ALI) is a more realistic approach than exposures of submerged cells. The published literature, however, describes inconsistent and/or extreme experimental conditions using ALI systems. We have compared five ALI systems for their ability to deliver both particulate matter (PM) and gases to cells cultured on porous membrane inserts. The ALI systems use different mechanisms to deliver pollutants to the inserts: diffusion, sedimentation, electrostatic precipitation (ESP), and thermophoresis (THP). We used fluorescent polystyrene latex spheres (PSLs) as a surrogate for PM to assess the efficacy of particle deposition in each system. PM loading in each insert was determined by dissolving the PSLs in ethyl acetate and measuring the fluorescence. Results show that using ESP as an external force enhances deposition of 50-nm PSLs by 5.5-fold and 11-fold for 1-µm PSLs when compared to diffusion alone. Similarly, THP enhances deposition of 50-nm and 1-µm PSLs by 4.5-fold and 2.7-fold, respectively. The interaction of ozone with an indigo dye on the surface of the insert showed that diffusion alone permitted gas-cell interaction. For each system there were various design and operational factors, such as the flow rate, surface materials and flow path geometry that adversely affected performance. Increased flow rates correlated with increased efficacy of the systems to deliver the gas to the inserts. Our results provide insights as to why inconsistent or extreme experimental conditions have been used with similar ALI exposure systems. [Abstract does not reflect the policies of the EPA.]

P20

Mechanism of rotenone mitotoxicity in *C. elegans*: role of the glyoxylate pathway

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We evaluated the effect of the pesticide rotenone, a known mitochondrial complex I inhibitor, on mitochondrial respiration (using the Seahorse XFe bioanalyzer), targeted metabolomics and ATP production using the nematode *C. elegans*. Our results show no effect of rotenone on mitochondrial parameters such as spare capacity, proton leak or ATP-linked respiration, or on basal oxygen consumption rate. We had previously observed no effect of rotenone on the number of mitochondrial genome copies or damage to mitochondrial DNA; opposite results have been published using mammalian systems and *C. elegans* at different life stages. Targeted metabolomics showed increases in organic acids (lactate, pyruvate, malate) and the amino acid alanine, and major decreases in long chain acylcarnitines after exposure to rotenone. We hypothesize that *C. elegans* might be able to handle the effects of rotenone exposure by bypassing the complex I inhibition it causes; the increase in malate suggests that the nematodes might be using the glyoxylate pathway as a way to bypass complex I. To explore this, we evaluated gene expression of the glyoxylate pathway enzyme isocitrate lyase (gene *icl-1*), and complex II subunits *mev-1* and *sdha-1*). All three genes showed an induction equal or greater than 1.4-fold change compared to control. ATP measurements indicate an increase in complex II function after exposure to rotenone; however, no change in function is apparent for complex I. Our results, although inconclusive, suggest that the effects of the glyoxylate pathway should be considered when working with complex I inhibitors in adult *C. elegans*.

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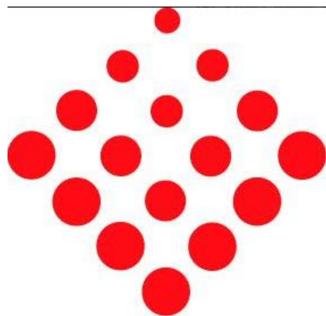

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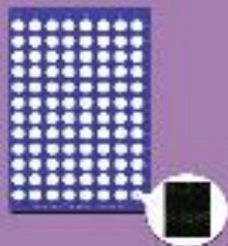

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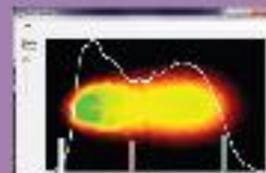

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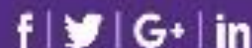

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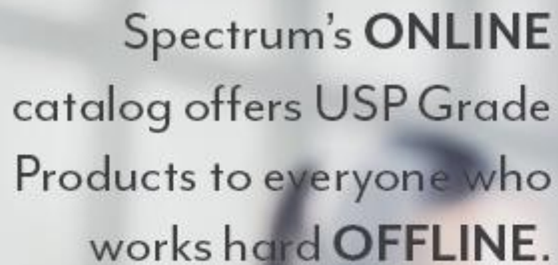

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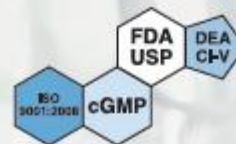

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