Genetics and Environmental Mutagenesis Society

35th Annual Fall Meeting

Human Genetic Susceptibility to Environmental Toxicants

Tuesday, November 7th, 2017

NC Biotechnology Center
15 T.W. Alexander Drive
Durham, NC 27703

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PRESIDENT'S MESSAGE

Dear GEMS members,

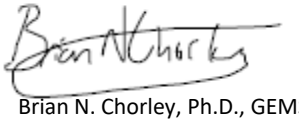
Welcome to our 35th Annual Fall Meeting! It is hard to believe that for 35 years the Genetics and Mutagenesis Society (and formally the Genotoxicity and Environmental Mutagenesis Society) has been hosting the Fall meeting! One of foundational principles of GEMS is to provide trainees and technicians – those who do the heavy lifting in the lab – a local forum to present their work and discuss their science. The Annual Fall Meeting was created specifically to foster this interaction and is a central activity of our Society. It is a testament to our members, sponsors, and those who participate in generating the *science*, that this tradition has continued for so long.

This year, our President-Elect and Program Chair, Holly Mortensen, has selected a highly relevant and timely topic, "Human Genetic Susceptibility to Environmental Toxicants". This subject is certainly on the mind of regulators at the US Environmental Protection Agency, as the recent update to the Toxic Control Substances Act (TSCA) has mandated the Agency consider susceptibility as a factor to toxicant exposure. Beyond the mandate, genetic susceptibility has been of scientific interest since the time of Mendel and certainly is central to current research, in particular toxicological studies. Understanding genetic mechanisms of disease susceptibility like these, and the interaction with environment, we can better consider susceptible subpopulations under a regulatory context. Today's meeting will highlight these important issues.

It has been my great pleasure to serve as the Society's President for 2017. I was humbled by the nomination and election by our members and I worked hard to continue the outstanding work of past Presidents to build and promote GEMS. My primary effort was to enhance this promotion by establishing Internet Communication, Social, and Finance committees within the Board of Directors. Under the direction of Cathy Sprankle and Caren Weinhouse, GEMS has entered the world of social media, which provides a new way for us to communicate with our members and those you are interested in our Society. You can now check GEMS out on Facebook, Twitter, and LinkedIn. It is the best way to keep up to date with activities, meetings, and science information! In addition, we have also begun to host more social activities to bring together members and prospective members in a relaxed and collegial environment. I thank Natalia Ryan and Jenna Guynn for leading these efforts this year. Finally, the finance committee, led by our Treasurer, Lisa Smeester and Past President, Tom Hughes, have helped secure our investments and grow our treasury so that we can support future GEMS activities.

Lastly, I'd like to thank all those who have supported GEMS over the past year. We cannot do what we do without our sponsorship. I, in particular, would like to recognize the support of NIEHS, EMGS, and Burroughs-Wellcome Fund, who, with their support, have allowed us to host our meetings and support the trainees with cash and travel awards. For example, our 2016 Best Talk and EMGS Travel Award supported Dr. Colette Miller to attend the 2017 EMGS in Raleigh, NC this year. Our Officers and Counselors -- Holly Mortensen (President-Elect), Nisha Sipes (Secretary), Lisa Smeester (Treasurer), and Counselors Tom Hughes, Cathy Sprankle, Natalia Ryan, Jenna Currier, Caren Weinhouse, Nagu Keshava, Michelle Campbell, Janice Lee, and Erin Hines – are a hard-working and dedicated group, and have my job as President easier! I also would like to thank Kristine Witt, who has helped obtain for many years our awards and mementos to hand out during the meetings, and to Carol Swartz who has been our long-time Corporate Sponsor guru (and now our newly elected President-Elect!). I also welcome aboard Michelle Angrish, Allison Harrill, and Natalie Saini as our new GEMS Counselors!

Thank you again for attending the meeting! Please continue to support our Society by sustaining membership, participating in our events, and communicating your great science through our new social media channels! Enjoy!



Brian N. Chorley, Ph.D., GEMS President (2016-1017)

AGENDA

8:00 - 8:45am	**Registration and Continental Breakfast**
8:45 – 9:00am	**Welcome** Brian Chorley, Ph.D., President Holly Mortensen, Ph.D., President-Elect
9:00 – 9:45am	**Screening for Population Susceptibility to Environmental Chemicals using DO Mouse Cells and High-Throughput Transcriptomics with the S1500+ Platform** **Rick Paules, Ph.D.**, NIEHS, RTP, NC

9:45 – 10:30am	**Assessing inter-individual variability with high-throughput transcriptomics** **John House, Ph.D.**, North Carolina State University, Raleigh, NC
10:30 – 11:30pm	**Poster Session and Sponsor Exhibits** 10:30 – 11:15: Odd posters attended 11:15 – 12:00: Even posters attended
11:30 –12:15pm	**TSCA Reform and EPA Requirements in Evaluation of Risk-Based Safety in Consideration of Vulnerable Populations** **Tala Henry, Ph.D.**, US EPA, OPP, Washington, DC
12:15 – 1:15pm	**Lunch (provided with registration)** Business Meeting
1:15 – 2:00pm	**How Data Science Can Inform Environmental Justice and Community Risk Screening of Genetic Susceptibility** **Ingrid Druwe, Ph.D.**, US EPA, NCEA, RTP, NC
2:00 – 2:45pm	**Trainee Platform Presentations**
2:45 – 3:15pm	**Break (beverages and snacks)**
3:15 – 4:00pm	**Identifying Susceptible Populations Using Exposure and Toxicokinetics** **John Wambaugh, Ph.D.**, US EPA, NCCT, RTP, NC
4:00 – 4:30pm	**Awards and Closing Remarks; Adjourn at 4:30pm**

MEET OUR INVITED SPEAKERS

Richard S. Paules, Ph.D.

Acting Chief, Biomolecular Screening Branch
Division of the National Toxicology Program
National Institute of Environmental Health Sciences

Richard S. Paules, Ph.D., is the Acting Chief of the Biomolecular Screening Branch (BSB) in the Division of the National Toxicology Program at the National Institute of Environmental Health Sciences, NIH. The Biomolecular Screening Branch

develops and carries out programs in medium and high throughput screening of environmental substances for rapid detection of biological activities of significance to toxicology and administers NTP programs designed to implement its vision for toxicology in the 21st century. In support of this program, BSB members represent the NTP in the U.S. Toxicology in the 21st Century, or Tox21, Federal Collaboration between members of the NTP, U.S. EPA, U.S. FDA and the National Center for Advancing Translations Science (NCATS) at NIH. The research interests of Dr. Paules include integrating conventional studies of environmental exposures and toxicity with novel model systems to rapidly assess exposure effects, including global systems or "omics" approaches, or *toxicogenomics*. He also holds adjunct faculty appointments as Professor in the Department of Pathology and Laboratory Medicine and Member in the Lineberger Comprehensive Cancer Center at the University of North Carolina at Chapel Hill. Dr. Paules received his Ph.D. from the Department of Pathology at UNC-CH and then received postdoctoral training with George F. Vande Woude at the National Cancer Institute, NIH, before joining NIEHS in 1990. He has authored over 100 peer-reviewed articles in leading biomedical journals, as well as 18 book chapters and invited publications. Since joining the NIEHS, he has been recognized with several awards, including four NIH Merit Awards and an NIH Director's Award, as well as the Society of Toxicology's *Leading Edge in Basic Science Award* at the 2010 SOT Annual Meeting.

John House, Ph.D.


Research Scholar
North Carolina State University

Trained as a molecular toxicologist, my past and current scientific work (and passion) resides at the intersection of human health, genetics, and the environment. My initial scientific discoveries were elucidating the roles of CCAAT/Enhancer Binding Proteins (CEBPs) in cell death and differentiation, in homeostasis of the epidermis and in how they function in stratified squamous differentiation. This work led to the discovery that these two transcription factors are essential in the terminal differentiation and homeostasis of sebaceous glands. My work with Stephanie London provided causal evidence for human GWAS identified SNPs implicated in pulmonary function. In addition, I showed in a subset of the Agricultural Health Study cohort that pre-natal and early life farming exposures are associated with lifelong protection of atopic sensitization and pulmonary function. In the past two years, my research has focused on data science; specifically on expression, genetics, epigenetics and epidemiology. My current research focus is two-fold: 1) pipeline and method development for evaluation of dose response and chemical characterization (read across) and 2) the roles of maternal and child diet on neuro-behavioral outcomes in children.

Tala Henry, Ph.D.

Director, Risk Assessment Division
US EPA

Ingrid Drewe, Ph.D.


Integrated Risk Information System
National Center for Environmental Assessment
US EPA

Ingrid L Druwe is a Toxicologist at the US Environmental Protection Agency's (EPA) National Center for Environmental Assessment (NCEA) Integrated Risk Information System (IRIS) Divison. Dr Druwe's expertise ranges from understanding of stimulation and perturbation of underlying mechanisms of action by a wide range of environmental chemicals to the mechanisms underlying disease processes such as cardiovascular disease, diabetes, and various cancers. She has used her expertise in molecular biology to develop adverse outcome pathways (AOPs) to help support chemical risk assessments for the Integrated Risk Information System (IRIS) at US EPA. Dr Druwe has combined her expertise in the development of High Throughput Assays (HTP) to screen potential developmental neurotoxicants (DNTs) and Bayesian statistical methods to develop Bayesian Methods for integration of HTP data to help prioritize chemical assessments of data poor environmental chemicals for EPA NCEA.

Prior to joining EPA NCEA IRIS Dr Druwe received her BS in Chemistry from Northeastern Illinois University in Chicago, Il. She then attended the University of Arizona where she studied the diabetogenic effects of arsenic and earned her doctorate degree in Pharmacology & Toxicology under the guidance of Dr. Richard R. Vaillancourt. She was the recipient of an NIH Ruth R Kirschstein National Research Service Award (NRSA). Her research also earned the University of Arizona College of Pharmacy Caldwell Award. After earning her doctorate degree Dr Druwe joined Dr. Bill Mundy's group at the US EPA National Health Effects and Exposure Laboratory as postdoctoral fellow through a cooperative agreement with the University of North Carolina- Chapel Hill. Under Dr Mundy's guidance, Dr Druwe developed high throughput (HTP) screening assays to screen potential developmental neurotoxicants. She earned the Cellular Dynamics Innovative Research grant award in 2013 for her work using Induced Pluripotent Stem cells to screen chemicals for hazard prioritization. She joined the US EPA National Center for Environmental Assessment (NCEA) as an ORISE postdoctoral fellow in the Fall of 2014. As a postdoctoral trainee Dr Druwe worked on the Arsenic IRIS assessment and developed AOPs for various Adverse Outcomes related to arsenic exposure. She has also worked on developing and incorporating Bayesian methods to integrate high throughput data for chemical risk prioritization.

Dr Druwe is a member of the Society of Toxicology (SOT) and a member of various specialty sections within SOT, such as the Risk Assessment Specialty Section (RASS) and the Hispanic Organization of Toxicologists (HOT). Additionally, she serves as the Vice Chair of the Dose Response Specialty Section for the Society of Risk Analysis.

John Wambaugh, Ph.D.


National Center for Computational Toxicology, US EPA

John Wambaugh is a Physical Scientist with the National Center for Computational Toxicology (NCCT). John's areas of active research include high throughput methods for exposure, toxicokinetics, and toxicology. He co-leads the EPA Rapid Exposure and Dosimetry (RED) project, which supports "exposure forecasting" or "ExpoCast" research. John is also a member of the ToxCast research team. John Wambaugh received his Ph.D. in physics from Duke University in 2006. John trained as a post-doctoral researcher with NCCT, where he studied pharmacokinetics and statistical analysis of biological models with an emphasis on Bayesian methods and integrating multiple data types.

MEET OUR INVITED SPEAKERS

ABSTRACTS

Short Talks

T1

Exploring the Role of Host-associated Microbiota as Mediators of Bisphenol Chemical Toxicity in Zebrafish

Tara R. Catron[1], Drake Phelps[1], Scott Keely[2], Nichole Brinkman[2], Emily Anneken[2], Allison Kvasnicka[3], Charles Wood[4], Shaza Gaballah[1], and Tamara Tal[4]

[1]ORISE/US EPA/ORD/NHEERL/ISTD, RTP, NC 27711; [2]US EPA/ORD/NERL/SED, Cincinnati, OH 45220; [3]Meredith College, Raleigh, NC 27607; [4]US EPA/ORD/NHEERL/ISTD, RTP, NC 27711

Exposure to Bisphenol A (BPA), a widespread environmental contaminant, has been associated with adverse endocrine and neurodevelopmental effects. Because host-associated microbiota play important roles in neurodevelopment and may bioactivate or detoxify xenobiotics, we hypothesized that developmental exposure to bisphenol compounds may influence microbiota community structure leading to colonization-dependent neurotoxicity. Conventionally colonized (CC) zebrafish were exposed to BPA, Bisphenol AF (BPAF), Bisphenol B (BPB), Bisphenol F (BPF), or Bisphenol S (BPS). The classic estrogen receptor agonist 17beta-estradiol (E2) served as a positive control. At 10 days post fertilization (dpf) larvae were assessed for mortality and a range of potencies were observed: BPAF > E2 > BPB > BPA > BPF > BPS. To evaluate potential chemical-dependent shifts in microbiota structure, 16S rRNA gene sequencing was performed. Concentration-dependent microbiota disruption was observed with BPA, BPF, and BPS exposure. BPAF, BPB and E2 exposure did not alter microbiota. These data demonstrate that the least overtly toxic bisphenol compounds cause the most significant alterations in microbiota structure. To determine if neurobehavioral toxicity was mediated by microbiota, three zebrafish cohorts were exposed to each chemical: CC, axenic (microbe-free), and axenic colonized with zebrafish facility water at 1 dpf. At 10 dpf, neurobehavioral effects were assessed using an established light/dark assay. Colonization-dependent hypoactivity was observed only with E2 exposure. While some BP compounds caused neurotoxicity, the effect was not colonization-dependent. These differential effects suggest that current hazard identification strategies may misestimate risk if chemical-microbiota interactions are not considered. *This abstract does not necessarily reflect EPA policy.*

T2

Smoking-associated *AHRR* hypomethylation enables cell-type specific enhancer activation and gene expression

Ma Wan[1], Brian D. Bennett[2], Gary S. Pittman[1], Michelle R. Campbell[1], Lindsay M. Reynolds[3], Devin K. Porter[1], Chris L. Crowl[1], Xuting Wang[1], Dan Su[1], Neal A. Englert[1], Yongmei Liu[3], and Douglas A. Bell[1]

[1]Environmental Epigenomics and Disease Group, IIDL, NIEHS, 27709; [2]Integrative Bioinformatics Group, NIEHS, 27709; [3]Department of Epidemiology and Prevention, Wake Forest School of Medicine, 27157

While tobacco smoke exposure strongly influences DNA methylation and is causative in numerous human diseases, the underlying mechanistic links are obscure. We investigated genome-wide smoking-associated differentially methylated regions (SM-DMRs) using CD14 monocytes of smokers (n=47) and nonsmokers (n=46) from two independently recruited populations. SM-DMRs preferentially occur at genomic regions with the characteristics of putative enhancer, open chromatin, and the enrichment of TF binding regions. Most of our selected candidate SM-DMRs identified in the two populations were also observed in other hematopoietic cell types and were successfully validated using a second method in an independently recruited group of subjects. Aryl-Hydrocarbon Receptor Repressor (*AHRR*) SM-DMR, located at an intragenic enhancer, was the most significantly affected DMR and we have previously reported that a methylation level of a CpG in this gene was associated with subclinical atherosclerosis. The *AHRR* DMR was also detected in saliva DNA and these results were highly correlated with effects in monocytes ($r^2 = 0.90$), suggesting that saliva may provide a potential alternative, noninvasive source for biomarkers that use DNA. Mechanistically, our results suggested that *AHRR* SM-DMR upregulated *AHRR* mRNA through activating *AHRR* enhancer in monocytes of smokers but not in granulocytes, indicated by increased noncoding RNA. In line with these findings, our preliminary data shows that overexpression of human *AHRR* in monocytic THP-1 cells resulted in significantly upregulation of genes involved in inflammation, the major cause of atherosclerosis. Taken together, our study suggests that cell type-specific activation of enhancers at SM-DMRs may represent a mechanism driving smoking-related disease.

T3

Arsenic (+3 oxidation state) Methyltransferase (*AS3MT*) Genotype is Associated with Metabolites that are Linked to Diabetes Susceptibility in Individuals Exposed to Arsenic in Chihuahua, Mexico

Elizabeth M. Martin[1], Carmen González-Horta[2], Blanca Sánchez-Ramírez[2], Lourdes Ballinas-Casarrubias[2], María C. Ishida[2], Daniela S. Gutiérrez -Torres[2], Roberto Hernández Ceron[3], Damián Viniegra Morales[3], Francisco A. Baeza Terrazas[3], Zuzana Drobna[5], Wei Jia[6], Gonzalo G. García-Vargas[7], Luz M. Del Razo[8], Michelle A. Mendez[4], Miroslav Styblo[4], and Rebecca Fry[1]

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The enzyme Arsenic-3-Methyltransferase (AS3MT) is responsible for the metabolism of inorganic arsenic (iAs). Polymorphic variants of *AS3MT* are associated with increased risk of iAs-associated diseases including diabetes mellitus (DM). At present, it is not clear what metabolic processes *AS3MT* genotype influence that impact the risk of diabetes development in the context of iAs exposure. To investigate the role of *AS3MT* polymorphisms as a susceptibility factor in the development of DM, we integrated genotype and metabolomics data from diabetic and non-diabetic individuals exposed to iAs. The 123 profiled individuals (56 diabetic individuals and 67 non-diabetic individuals) come from our Chihuahua Cohort in Mexico, where arsenic levels in drinking water range from <1-292.9 µg/L. To characterize metabolite profiles in relation to six single nucleotide polymorphisms (SNPs) in *AS3MT* linear regression modeling was used. The results demonstrate that four SNPs, rs17881215, rs3740393, rs3740390 and rs10748835, were associated with 12 urinary and plasma metabolites. Among the identified metabolites, an enrichment for, amino acid metabolism was identified. Interestingly, the enriched metabolites included phenylalanine, arginine and methyl-L-histidine which have been previously linked to blood sugar control in humans and delay of suppression of liver gluconeogenesis. This novel integration elucidates a potential mechanistic basis, increased blood sugar

control and suppressed liver gluconeogenesis, for the findings that *AS3MT* polymorphisms are associated with risk of DM development in iAs exposed populations.

Poster Abstracts

P1

Exome Sequencing of Spontaneous and Chemically-induced Hepatocellular Carcinomas in B6C3F1/N Mouse Identifies Unique Somatic Mutations

Miaofei Xu[1], Scott Auerbach[1], Mark Hoenerhoff[1,2], Alex Merrick[1], Dhiral Phadke[3], Ruchir Shah[3], Hue-Hua Hong[1], Ramesh Kovi[1,4], Robert Sills[1], and Arun Pandiri[1]

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Introduction: Hepatocellular carcinoma (HCC) is the third-leading cause of cancer-related death in the world. The B6C3F1/N mouse has a moderate background incidence of HCC and increased incidences due to chemical exposures are frequently observed in NTP bioassays. A better translational insight into the mouse HCCs can be gained from a comprehensive evaluation of the mutations in HCCs that arise either spontaneously or due to chemical exposures.

Experimental Design and Methods: Whole exome sequencing (WES) was performed on DNA extracted from fresh-frozen liver tissues of B6C3F1/N mouse HCCs, arising either spontaneously (n=3) or due to chronic exposure to carcinogens such as Gingko Biloba Extract (GBE) (n=4), and Methyl Eugenol (MEG) (n=3) as well as age-matched normal livers (n=3). The genomic sequence data from the parental strains C57BL/6 and C3H/HeJ and NCBI37/mm9 were used as the reference genomes for variant calling.

Results: Mutations in *Hras* (Q61K, Q61R) and *Ctnnb1* (D32N) were confirmed by Sanger sequencing. However, mutations in *Braf* (V637E) and *Bves* (R180S, P195L) obtained by WES were not confirmed in an independent group of 37 samples. The MEG-induced tumors showed a pattern similar to Signature 24 in the Catalogue of Somatic Mutations in Cancer (COSMIC) database.

Conclusion: Unique mutation spectra and mutated genes could serve as potential biomarkers of exposure and disease.

Impact statement: Etiology-dependent unique somatic mutations in mouse HCCs may potentially be used as biomarkers of exposure and neoplasia. Additional data from larger sample sizes will provide greater confidence in this data and approach.

P2

Genetic variation in the *AHRR* gene and smoking-associated hypomethylation

Isabel J.B. Thompson, Michelle R. Campbell, Gary S. Pittman, Xuting Wang, Ma Wan, and Douglas A. Bell.

Immunity, Inflammation, and Disease Laboratory, National Institute of Environmental Health Science, National Institute of Health, Research Triangle Park, NC 27709

Exposure to tobacco smoke is associated with a variety of health problems, including risk of cardiovascular and respiratory diseases as well as cancer. Previous studies have shown that DNA methylation level at CpG site cg05575921 in the AHRR gene is a reliable biomarker of tobacco smoke exposure. Recent research has suggested that genotype may mediate the effects of smoking on DNA methylation. We chose eleven single nucleotide polymorphism (SNPs) in *AHRR*, previously identified as eQTL SNPs in humans, to investigate the possible effect of genotype on smoking-induced methylation changes. Age, race, and sex matched smoking and nonsmoking volunteers provided blood samples and smoking history, and serum cotinine was determined. Methylation level for *AHRR* cg05575921 was determined for each subject and analyzed relative to smoking exposure and genotypes at AHRR. We used regression analysis to test the relationship between smoking exposure (log2 cotinine), genotype, and methylation level at cg05575921. Genotype at one SNP, rs11740668, was significantly associated with methylation level (p=0.008). When stratified by race, it was only significant in the white population. Including a 'genotype*smoking' interaction term in the model revealed a significant interaction between rs11746079, smoking status, and methylation of AHRR (p = 0.045). We observed a sharper decrease in methylation among smokers with the less common C allele than with the TT genotype. Future plans will explore if SNP genotype mediates the association between smoking exposure, *AHRR* DNA methylation and gene expression.

P3

Zebrafish Larvae Require Specific Strains of Bacteria to Allow for Normal Neurobehavioral Development

Allison Kvasnicka[1], Shaza Gaballah[2], Drake Phelps[2], Tara Catron[2], Nichole Brinkman[3], Scott Keely[3], Emily Anneken[3], Charles Wood[4], and Tamara Tal[4].

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There is an increasing appreciation of the relationship between gut microbiota and nervous system development and function. We previously showed that axenic (microbe-free) larvae are hyperactive at 10 days post fertilization (dpf) relative to colonized zebrafish larvae. Interestingly, while exposure to heat-killed bacteria or microbe-associated molecular patterns failed to block hyperactivity in axenic larvae, colonization of axenic larvae with *Aeromonas veronii* or *Vibrio cholerae* produced locomotor hypoactivity relative to colonized controls. These data suggest that there is a developmental requirement for certain types of microbes modulate host behavior. To address this hypothesis, eight bacterial isolates were obtained from 10 dpf conventionally colonized zebrafish larvae. 16S rRNA gene sequencing identified four unique gram-negative isolates: *Acinetobacter*, *Vibrio*, *Comamonas*, and Comamonadaceae. Colonization of axenic embryos at 1 dpf with 100 cells/mL of *Acinetobacter*, *Comamonas*, or *Comamonadaceae* resulted in behavioral profiles that were identical to colonized control larvae at 8 dpf. In comparison, axenic embryos colonized with *Vibrio* bacteria were hypoactive relative to control larvae. *Vibrio*-related hypoactivity was prevented in axenic larvae colonized with 25 cells/mL each of *Actinetobacter*, *Vibrio*, *Comamonas*, and *Comamonadaceae* at 1 dpf. Finally, *Vibrio*- related hypoactivity was found to persist in 10 dpf larvae. These data suggest that specific bacterial taxa are needed to drive normal neurobehavioral development while colonization with other strains may result in behavioral hypoactivity. These findings raise the possibility that environmental chemicals may disrupt neurobehavioral development by selecting for specific classes of host-associated microbes. *This abstract does not represent EPA policy.*

P4

Humanized, Transgenic Caenorhabditis elegans to Study CYP2E1-Induced Mitochondrial and Neurotoxicity

Jessica H. Hartman[1], Kacy L. Gordon[2], David R. Sherwood[2], and Joel N. Meyer[1]

[1]Nicholas School of the Environment, Duke University, Durham, NC 27708; [2]Department of Biology, Duke University, Durham, NC 27708

Cytochrome P450 2E1 (CYP2E1) is a mammalian enzyme that metabolizes small hydrophobic pollutants, drugs, and endogenous molecules. CYP2E1 metabolism results in detoxification and improved elimination or, paradoxically, bioactivation to reactive (toxic) metabolites. Understanding CYP2E1 metabolism and its consequences will therefore improve risk assessment for exposures to CYP2E1-activated compounds. Of particular interest to this project is mitochondrial toxicity and neurotoxicity associated with the volatile organic pollutants trichloroethylene and methanol, processes which we hypothesize are CYP2E1-dependent. To test this hypothesis, we generated novel *C. elegans* nematode strains that express human CYP2E1. Wild-type nematodes do not express CYP2E1, and therefore have no background activity, while our transgenic animals displayed robust CYP2E1 activity. CYP2E1 expression alone caused changes to mitochondrial morphology, manifesting in more fragmentation and disruption of mitochondrial networks compared to age-matched wild-type controls ($p<0.001$). Furthermore, a 48-hour exposure of adult CYP2E1-expressing nematodes to the classic CYP2E1-activated drug acetaminophen resulted in significantly more lethality (25% at 3mM, 50% at 25mM) compared to wild-type N2 nematodes, which did not show any lethality up to 25mM acetaminophen. By contrast, wild-type larval nematodes were sensitive to acetaminophen-induced growth delay, possibly due to disruption of developmental signaling, while CYP2E1-expressing nematodes were protected from this delay ($p<0.01$). Future and ongoing experiments will determine the relative sensitivity of CYP2E1-expressing nematodes to CYP2E1-activated mitochondrial toxicants methanol and trichloroethylene, including lethality and sublethal endpoints such as neurodegeneration and mitochondrial dysfunction. Ultimately, the results of this study will reveal the role of CYP2E1 in driving toxicant-induced mitochondrial dysfunction and neurodegeneration.

P5

AOP-DB Frontend: A user interface for the Adverse Outcome Pathways Database

Phillip H. Langley[1], Evgeniia Kazymova[1], Beena Vallanat[2], and Holly M. Mortensen[2]

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The EPA Adverse Outcome Pathway Database (AOP-DB) is a database resource that aggregates association relationships between AOPs, genes, chemicals, diseases, pathways, species orthology information, ontologies. The AOP-DB frontend is a simple yet powerful user interface in the form of a web application. By replacing applications like MySQL Workbench and the MySQL command line interface (MySQL CLI), it allows users to interact with the SQL database conveniently within their preferred web browser on any operating system. This approach includes all information the AOP-DB has to offer without the need of excess applications or knowledge of creating SQL queries. The frontend itself is built using pure-JavaScript frameworks Node.js and AngularJS. These modern frameworks allow for increased modularity, responsiveness, and robust page features without impacting development time. Currently, the AOP-DB frontend is envisioned to be utilized for exclusive in-house EPA use; though this is the case, the frontend is being built with eventual public external, internet use in mind by taking appropriate measures for administration and security. Alongside these considerations, the application will also serve as a framework for other databases housed at the EPA. The workflow described here includes a finished mockup for the AOP-DB being available April/May 2018. Here we describe the prototype, which includes essential features such as database navigation, and site administration. *This abstract does not reflect EPA Policy*

P6

Prediction of cellular mode-of-action based on computational modeling of high-throughput toxicogenomics data: a pathway enrichment approach with machine learning

Saad Haider, Kamel Mansouri, Michael B. Black, Patrick D. McMullen

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Interpretation of cellular modes-of-action after exposure to chemicals is conventionally performed by whole-transcriptome expression tools such as microarray and RNA-Seq. However, for the toxicity assessment of copious chemicals, efficient alternatives like high-throughput transcriptomics (HTT) are pressingly needed. Our goal was to develop suitable HTT-models for evaluating diverse cellular modes-of-action (MOA) of environmental, industrial, and agricultural compounds.

Genometry's L1000 platform was used to demonstrate the possibility for establishing statistical relationships between genes and to infer whole genome transcriptional profiles. We developed an MOA-oriented strategy to predict relevant cellular pathway information and applied it to Affymetrix array data collected from three human cell lines exposed to three agrichemicals over nine concentrations. We built three-class categorical models predicting up-regulated, down-regulated and unchanged genes essential to determining cellularMOA.

We then focused on predicting gene-expression changes resulting from exposure to heterogenous chemicals from a wide range of classes. For model fitting, we used the Open TG-GATEs toxicogenomics database containing multiple cell lines for over 900 samples covering ~170 compounds. Different machine learning techniques were used to identify landmark genes which can predict differential expression changes of the genomic profile. A 10-fold cross validation balanced-accuracy of ~65% was reached compared to a correlation coefficient of 42% with continuous models. The predicted and actual Affymetrix pathway enrichment profiles for an HepaRG cell line had the 22 (of 39) highest enriched pathways in common. Such models can be used for MOA prediction and pathway analysis to prioritize large libraries of chemicals starting from expression data of representative genes.

P7

Repurposing Archival Samples for Investigating Toxicological Modes of Action

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Little is known about formalin fixation induced genomic artifacts, limiting the use of formalin-fixed paraffin-embedded (FFPE) samples in toxicological and clinical studies. Previously, we identified a consistent shift in transcriptional profiles between paired frozen and FFPE samples, which we hypothesized was caused by fixation of fresh tissue formalin. To test this, liver samples were collected from male B6C3F1 mice treated with 600 ppm phenobarbital (PB) or vehicle control (Con) for 7 days. Samples were: 1) fresh-frozen (FR); 2) directly fixed in 10% buffered formalin for 18 hours and processed to FFPE (FIX); or 3) initially frozen (FR>FIX) and processed as FIX (n=6/group/condition). The FR>FIX group served as a technical control. Samples were sequenced on an Illumina Hi-seq 2500. Reads were aligned using Star (2.4) and analyzed in Partek Flow (6.0). FIX *vs*. FR resulted in 2946 differentially expressed genes (DEGs) (98% down-regulated). FR>FIX *vs*. FR resulted in 95% fewer DEGs, suggesting the formalin effect occurs at fixation. Comparative analysis of the formalin-induced DEGs with two independent studies in Ingenuity Pathway Analysis identified enrichment in oxidative stress, mitochondrial dysfunction and transcription elongation pathways. However, direct fixation did not clearly impact chemical response. PB treatment induced 180 DEGs within the FIX and 159 in FR>FIX group of which 120 were shared. DEGs were consistent with CAR/PXR activation and PB exposure, suggesting the formalin effect did not confound the chemical response. Our results will advance the use of FFPE samples for investigating chemical modes of action. This abstract does not reflect EPA policy.

P8

Performance Evaluation of High Throughput Transcriptome Extrapolation Techniques

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The Tox21 consortium is developing a low-cost, high-throughput transcriptome (HTT) assessment approach in which a predetermined subset of the transcriptome is measured to screen chemicals and characterize toxicity and disease pathways. Since only a small subset of the transcriptome is measured experimentally, this approach has the potential to greatly decrease the cost of toxicogenomic screening. The method relies on computational extrapolation techniques that are trained using large amounts of curated whole transcriptome profiles to precisely infer the unmeasured portions of the transcriptome by intelligently leveraging gene-to-gene interconnectedness. Here we present a comparison of two popular large-scale extrapolation techniques considered for use in this pipeline: principal component regression (PCR) and Deep Learning (DL) using a multi-layer, feedforward neural network. Raw signal for all unique (N=117,559) GPL570 microarray data files from NCBI GEO were downloaded and preprocessed in batch. Expression signal from 2,729 genes was used to extrapolate signal for 18,167 genes using each method with performance evaluated using 20-fold cross validation. Results indicate that PCR extrapolation outperformed DL extrapolation in terms of root mean square error (RMSE; PCR=0.39 vs. DL=0.51), median absolute error (MAE; PCR=0.20 vs. DL=0.26), and rank-biased overlap between the top 10% of true and extrapolated genes (RBO; PCR=66% vs. DL=58%). Additionally, PCR extrapolation is less computationally intensive, increasing its overall utility. Together, these results suggest that PCR extrapolation is a suitable method for use within HTT assessment pipelines and that it may be preferable to DL methods in this domain.

P9

Molecular Cloning Involving the AAV CXCL 12 Gene

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The American Cancer Society reports that this year there will be an estimated 600,920 deaths due to cancer in the United States. Current cancer research includes the use of biomarkers on the surface of cancer cells to distinguish the cancerous cells from normal body cells. Molecular cloning can enhance these biomarkers. Over the past thirty years, molecular cloning has progressed immensely. From digestion to plasmid insertion, the possibilities are endless. The AAV (Adeno Associated Virus) CXCL 12(C-X-C Motif Chemokine Ligand 12) is a Protein Coding gene that shows great promise with cloning and plasmid insertion. Our project aims to use this gene to bind tightly to biomarkers on the surface of cancer cells. However before this optimal binding can occur, it is essential to know more about the AAV CXCL 12 Gene itself. For this reason, our project includes multiple gel electrophoresis assays, plasmid insertion/digestion assays, and PCR purification. From the results of these assays, the efficacy of AAV CXCL 12 to bind to cancer biomarkers will become clear. In particular, the cloning assay for the AAV CXCL 12 gene holds great potential, as it is possible to clone extraneous DNA into a different host. If extraneous DNA can be cloned into a different host, then there is the possibility of that DNA binding to a biomarker on a cancer cell.

P10

Using Machine Learning and SWIFT-Active Screener to Reduce the Expense of Evidence Based Toxicology

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Evidence-based toxicology employs the rigorous methodology of Systematic Review to reach consensus about targeted questions concerning health effects of environmentally important chemicals. A critical and time-consuming step in this process is screening the available literature to select relevant studies. We have previously evaluated the performance of our web-based screening tool, SWIFT-Active Screener, using simulated screening of more than 100,000 abstracts from 20 different systematic reviews. Compared to traditional screening, our approach, which employs a machine learning method called "Active Learning" to priority rank abstracts, resulted in an average 54% reduction in screening burden, while still achieving 95% recall or higher; on the 13 studies containing >1,000 articles, the savings improved to 71%. Here, we extend this work using several recent systematic reviews conducted with our software by organizations such as NIEHS, EPA, USDA, TEDX and EBTC. Results from these "real world" data sets are similar to results previously observed in simulation. For example, in the largest of the projects evaluated, 20,883 references were dual-screened using Active Screener, with 99% recall achieved after screening only 42% of the total collection. Since each abstract took, on average, 1 minute per screener, the savings for this review alone was approximately 400 hours. More than 4,000 systematic reviews are performed annually in the fields of environmental health and evidence-based medicine, with each review averaging between six months to one year of effort; therefore, we anticipate that machine learning screening tools like Active Screener can help to greatly reduce the total cost of evidence-based toxicology.

P11

Establishing a Cell-based Assay for Assessment of Cellular Metabolism on Chemical Toxicity

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A major drawback of current *in vitro* chemical testing is that many commonly used cell lines lack chemical metabolism. To help address this challenge, we have established a method for assessing the impact of cellular metabolism on chemical-based cellular toxicity. A commonly used human cell line with low endogenous metabolism (HEK293T) was engineered to overexpress cytochrome P450 monooxygenase (CYP) transgenes prevalent in human liver (CYP3A4, CYP2E1, CYP1A1, and CYP1A2). Each clone was mated to a unique DNA barcode. A cytotoxicity screen with individual clones was performed with 29 chemicals reported to be affected by CYP metabolism using CellTiter Glo (CTG, Promega). Of the 22 chemicals reported to be activated by CYPs, we measured only 6 that exhibited >10% cell number loss by one or more CYP overexpressing clones. Interestingly, 3 of the 7 chemicals that were reportedly detoxified by or inhibited CYP activity exhibited cytotoxic effects when compared to control cells. Specific CYP subtype metabolism not represented in this pilot screen may account for differences from reported results. Concurrently, we are assessing higher throughput methods (multiplexed digital drop PCR and targeted DNA detection) that measure clone-associated barcodes that serve as surrogates of clone cell number (i.e., cytotoxicity). Such methods will allow us to increase the number of CYPs assessed in a single measurement. Ultimately, these methods will provide a rapid chemical screen that is more biologically relevant and will advance chemical safety assessment. *This abstract does not necessarily reflect US EPA policy.*

P12

Computational Integration of Human Genetic Data to Evaluate AOP-Specific Susceptibility

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There is a need for approaches to efficiently evaluate human genetic variability and susceptibility related to environmental chemical exposure. Direct estimation of the genetic contribution to variability in susceptibility to environmental chemicals is only possible in special cases where there is an observed association between exposure and effect (e.g. genotype and phenotype information). The availability of genetic and toxicological data sources makes it possible to indirectly estimate the relative contribution of genetic variability to differential human susceptibility. We developed a computational workflow that integrates genetic and toxicological resources. This approach implements the Adverse Outcome Pathway (AOP) framework in order to integrate molecular targets associated with AOPs with functional genomic annotations and population allele frequencies. Resources include the EPA internal Adverse Outcome Database (AOP-DB), and publicly available resources, such as the AOP-wiki, Ensembl genomic annotations, expression Quantitative Trait loci identified by the GTEx consortium, and 1000 Genomes Project. With this information it is possible to formulate predictions of genetic susceptibility built upon established toxicological and genetic knowledge that are specific to an adverse outcome.

The computational workflow was developed in R and built around the Ensembl database interfaces (REST API and biomaRt R package). It downloads, integrates, and analyzes the available data sources when an AOP is given as input. Data is processed in four steps: 1. Genetic identities of AOP key events are extracted from the AOP-DB; 2. Loss-of-function and nearby regulatory annotations are downloaded from Ensembl databases; 3. GTEx Expression quantitative trait loci are imported for AOP-relevant tissue types; and 4. Allele and haplotype frequency information is retrieved from the 1000 Genomes Project stage 3 dataset. Optionally, interfacing with AOP-DB also allows integration of chemical-gene and disease-gene interactions. The workflow includes a step for estimation of the degree of genetic variation at functionally relevant loci. With ongoing AOP development, this automated workflow will allow rapid assessment of outcome specific human genetic susceptibility. *This abstract does not reflect EPA Policy.*

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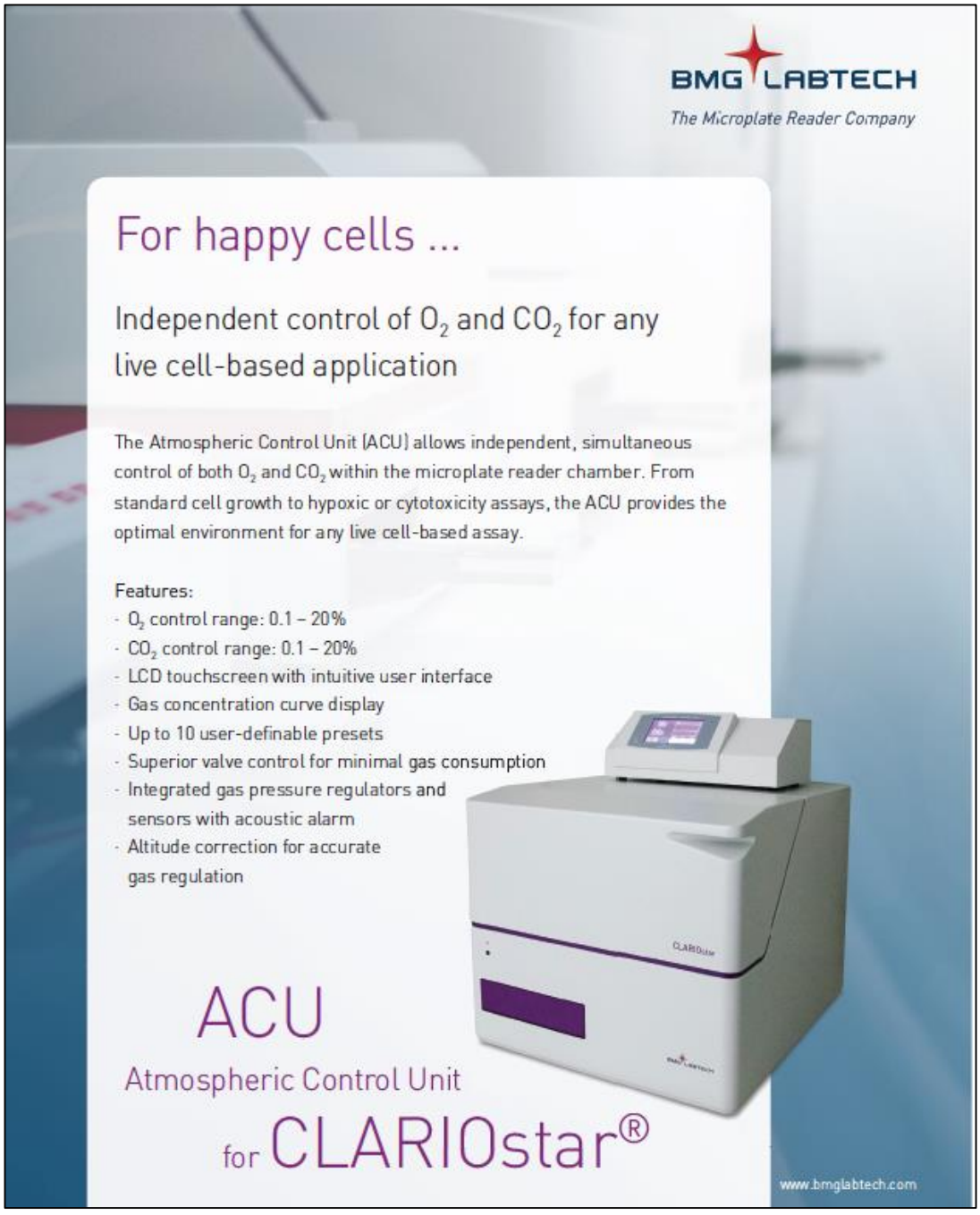
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NOTES