



32nd Annual Fall Meeting

**“Innovations and Integration of Genomics Data
to Advance Risk Assessment”**

Wednesday, October 22, 2014

North Carolina Biotechnology Center
15 T W Alexander Dr
Durham, NC 27703

Supported in part by grants from NIEHS.



National Institute of
Environmental Health Sciences

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October 22, 2014

PRESIDENT'S MESSAGE

Dear GEMS Members,

Welcome to the annual fall meeting of GEMS. Channa Keshava has put together an exciting program that addresses the application of 'omics' technologies to risk assessment. I am sure that we will leave the meeting with a much better appreciation of how this technology can be exploited to protect communities against environmental disease.

I have enjoyed my term as president of GEMS and thank you for giving me the opportunity. It has been a great pleasure working with the board of directors through our monthly meetings to sustain the society's mission. The spring symposium on "big air" was great success and I especially appreciated hearing about the scientific basis for classifying PM as a human carcinogen. Special thanks to David DeMarini for leading the organization of the spring meeting. Of course, Channa has served the society well making all the arrangements for the spring and fall meetings. I am sure he will be happy next year as President to sit back and let the next President-elect do the heavy lifting.

Our BOD is the heart of GEMS and could not function properly without the selfless effort of its many members. We should especially thank our secretary Holly Mortensen, for keeping the minutes and maintaining an archive of GEMS records, and our treasurer Jeff French, for keeping our finances in order. Our society functions largely through the effort of William Ward who provides much needed web support to keep our web media up-to-date and useful to us.

GEMS continues to function and serve our community in the face of reduced budgets in our many institutions. Monetary pressures represent a constant concern of the BOD but we have a system in place to keep yearly costs as low as possible and preserve our financial reserves. It is important that members show their support with payment of dues and attendance at meetings.

Finally let me report that GEMS awarded Dr. Jonathan Hall of NCSU, for last year's best oral presentation, \$500 for travel to the 2014 EMGS meeting in Orlando, FL. EMGS continues to provide an additional \$500 a year to our awardee for attendance at their meeting. I look forward to the oral presentations and posters by students and post-docs and congratulate this year's award winners.

I wish everyone a happy day. Enjoy the meeting.

Bill Kaufmann
GEMS, president

PRESIDENT ELECT (PROGRAM CHAIR'S) MESSAGE

Dear GEMS Members, Postdocs, Students and Guests,

With great pleasure, I welcome you to an exciting 32nd Annual Fall Meeting of the Genetics and Environmental Mutagenesis Society. The theme of this meeting is “**Innovations and Integration of Genomics Data to Advance Risk Assessment**”.

The field of genomics has expanded considerably over the past 10-15 years. New methodologies have been developed, validated and applied to screening of chemicals. These new innovations have assisted on integration of genomics data to advance risk assessment. This meeting will extensively discuss the applications of genomics in terms of forecasting chemical effects in biological systems, comparative genomic analyses, understanding the next generation risk assessments in terms of assessing population genetic variations using in vitro systems, and identifying adverse outcome pathways and biomarkers that bridge high throughput screening of data with regulatory decision making.

Dr. Scott Auerbach from NIEHS will start of discussing how the capability of the new technologies have resulted in our ability to comprehensively query the molecular domains of biology, when integrated at a systems level, would give rise to higher order of phenotypes measured in toxicology. Dr. Scott will provide specific examples of applications of omics-based hazard level characterizations.

Dr. Rebecca Fry from University of North Carolina will continue with the similar concept of identification of perturbed biological pathways that are associated with chemical exposure, providing comparative genomic analysis. Dr. Fry will specifically discuss regulators of the metals-induced pathways and application of their targets for disease prevention.

With the implementation of REACH and reduction of animal use in toxicology experiments, next-generation in vitro assays are providing important data for chemical prioritization and establishing benchmarks for risk due to exposure. **Dr. Fred Wright** from NC State University will discuss the importance of novel techniques for identifying constitutional vs technical variation in a population, heritability and individual genetic variants contributing to susceptibility.

Dr. Stephen Edwards from U.S. EPA will discuss Adverse Outcome Pathway (AOP) concept as an ideal framework for combining high throughput screening (HTS) approaches and computational models requiring sophisticated data sources to complement the HTS results. Dr. Edwards will provide recent information about the AOP knowledgebase that has been developed to organize and disseminate AOP-related information. This framework has been an international effort with scientists from different areas of research.

In addition to the Key note speakers, we will have four young investigator/Post-doc talks and several posters submitted by students and post-docs. I strongly believe that this is an excellent meeting with discussion on latest topics in toxicology and risk assessment.

I look forward to seeing you all at the meeting.

Channa Keshava,
GEMS President-elect



Innovations and Integration of Genomics Data to Advance risk Assessment

8:00am - 8:45am	Registration and Continental Breakfast
8:45am - 9:00 am	Welcome: Bill Kaufman , Ph.D., GEMS President Introductions: Channa Keshava , Ph.D., GEMS President-elect
9:00am - 9:45am	The Application of Toxicogenomic Compendium Data to Forecasting Chemical Effects in Biological Systems and Risk Assessment <i>Scott Auerbach</i> , Ph.D., Molecular Toxicology and Informatics Group, NIEHS, RTP, NC
9:45am - 10:30am	Comparative Genomic Analyses for the Identification of Disease Pathways <i>Rebecca Fry</i> , Ph.D., Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, NC
10:30am - 11:45am	Posters, Sponsor exhibits
11:45 - 1:00pm	LUNCH (provided)
1:00pm - 1:45pm	NexGen Risk Assessment - Prospects for Assessing Population Genetic Variation using In Vitro Assays <i>Fred Wright</i> , Ph.D., Department of Statistics, North Carolina State University, Raleigh, NC
1:45pm - 2:45pm	Talk by post docs/students 1:45 - 2:00 - talk 1: Renee A. Beardslee, NCSU, Raleigh, NC 2:00 - 2:15 - talk 2: Kin Chan, NIEHS, RTP, NC 2:15 - 2:30 - talk 3: Natalia VanDuyn, NHEERL, US EPA, RTP, NC 2:30 - 2:45 - talk 4: Michelle M. Angrish, US EPA, RTP, NC
2:45pm - 3:00pm	Break (beverages and snacks)
3:00pm - 3:45pm	AOPs & Biomarkers: Bridging High Throughput Screening and Regulatory Decision Making <i>Stephen Edwards</i> , Ph.D., National Health and Environmental Effects Research Laboratory, EPA, RTP, NC
3:45pm - 4:30pm	Business meeting, Announcement of Awards etc.
4:30pm	Adjourn



**Genetics and Environmental Mutagenesis Society
Fall Meeting**

October 22, 2014

North Carolina Biotechnology Center, RTP, NC

**“Innovations and Integration of Genomics
Data to Advance Risk Assessment”**

Speakers:



Scott Auerbach, Ph.D., Molecular Toxicology and Informatics Group, National Institute of Environmental Health Sciences, RTP, NC



Rebecca Fry, Ph.D., Environmental Sciences & Engineering, University of North Carolina, Chapel Hill, NC



Fred Wright, Ph.D., Department of Statistics, North Carolina State University, Raleigh, NC



Stephen Edwards, Ph.D. National Health and Environmental Effects Research Laboratory, Environmental Protection Agency, RTP, NC

SPEAKERS

Scott Auerbach, Ph.D. Molecular toxicology and Informatics Group, National Institute for Environmental Health Sciences, RTP, NC

Biosketch: Dr. Auerbach received a dual BS from The Pennsylvania State University in Physiology and Biochemistry/Molecular Biology in 1998. He then went on to receive his Ph.D. in Pharmacology from the University of Washington in 2004. From 2005 to 2007 Dr. Auerbach was a postdoctoral fellow at Duke University and then NIEHS under the direction Dr. David Schwartz where he undertook genetic studies of human pulmonary fibrosis. Subsequently he went on to a fellowship at the National Toxicology Program. Dr. Auerbach became a staff scientist at the National Toxicology Program in 2009 and became a Diplomat of the American Board of Toxicology the same year. His research focuses on application of molecular and high dimensional data to toxicological assessment with the goal of increasing efficiency in toxicology testing.

Abstract: The Application of Toxicogenomic Compendium Data to Forecasting Chemical Effects in Biological Systems

Over the last 15-20 years a number of technologies have been developed that have the ability to comprehensively query the molecular domains of biology. These domains, when integrated at a systems level, give rise to higher order phenotypes that have been measured in toxicology and medicine since the inception of these fields. Collectively these technologies are referred to as omics. The high dimensional data streams provided by omic technologies are both sensitive and comprehensive in their ability to capture biological perturbations. Importantly, these two latter characteristics are critical in chemical hazard characterization. Further these technologies are scalable and therefore have the potential to increase the productivity of chemical hazard characterization. At the NTP we have been exploring the use of transcriptomics and high throughput screening data for deriving both quantitative (*i.e.*, point of departure (POD)) and qualitative (the nature of the toxicity) information for formulating screening level hazard characterizations (HC). From a quantitative standpoint we have explored the use of gene, signature and pathway level POD characterization. In the case of qualitative toxicity characterization we have leveraged the feature rich aspect of omics data to perform nearest neighbor (NN) analysis within the context of toxicogenomic compendium data. Results from NN are analyzed using chemical annotation enrichment that forecasts toxicological/biological properties. The data, resources and methods that are being used in omics-based HC will be discussed. In addition, specific example applications of omics-based HC will be provided.

Rebecca Fry, Ph.D. Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, NC

Biosketch: Dr. Fry is an Associate Professor in the Department of Environmental Sciences and Engineering at the Gillings School of Global Public Health at UNC-Chapel Hill. She also holds appointments in the Curriculum in Toxicology and the Lineberger Cancer Center. Dr. Fry received her Ph.D. in Biology from Tulane University and completed her post-doctoral training in toxicogenomics/environmental health at MIT. She is the Deputy Director of UNC's Superfund Research Program and leads one of three of the biomedical research projects where she is investigating the effects of prenatal cadmium exposure on infant health in populations in North Carolina. She is also funded by the NIEHS to understand the health effects associated with prenatal arsenic exposure in a cohort in Mexico. Building off her expertise is in the areas of DNA repair, toxicogenomics and systems biology, her research at UNC focuses on mechanisms of disease associated with toxic metal exposure early in life. A primary goal of Dr. Fry's research is to increase awareness of the deleterious impacts of exposures during the prenatal period and to improve public health initiatives to address this issue.

Abstract: Comparative Genomic Analyses for the Identification of Disease Pathways

The identification of biological pathways that are perturbed by environmental toxicants is a critical component to understanding their mechanisms of action in disease causation. Of biological importance is the identification of key pathway-regulating-factors at both a transcriptional and translational level. These regulatory factors may be epigenetic as well as non-epigenetic in nature. Targeting the identified pathway regulators will be a key strategy for disease prevention and treatment. Here our research focused on the identification of perturbed biological pathways that are associated with toxic metal exposure is presented. The identification of the regulators of the metals-induced pathways discussed, and the application of their targeting for disease prevention highlighted. Taken together this information is integral for developing an integrated risk assessment framework.

Fred Wright, Ph.D. Department of Statistics, North Carolina State University, Raleigh, NC

Biosketch: Fred Wright is Professor of Statistics and Biological Sciences at NCSU and Director of the Bioinformatics Research Center. His activities have ranged from development of new methods of gene mapping to expression-quantitative trait (eQTL) mapping for multiple tissues, and for several years he has applied statistical genomics principles to long-standing problems in toxicology, with funding from NIH and the EPA. He is an elected Fellow of the American Statistical Association and the Delta Omega Honor society for Public Health.

Abstract: NexGen Risk Assessment – Prospects for Assessing Population Genetic Variation using In Vitro Assays

Next-generation in vitro assays are providing important data for chemical prioritization and establishing benchmarks for “average” risk due to exposure. Measuring the extent of population variation in susceptibility is also important, quantifying upper bounds on risk and potentially identifying susceptible subgroups. We use a cytotoxicity study of ~1000 lymphoblastoid cell lines and ~180 chemicals to illustrate the dissection of constitutional vs. technical variation, heritability and individual genetic variants contributing to susceptibility. These and similar studies show that variation in susceptibility from cell-based assays is a complex trait, with numerous genetic variants each responsible for a small effect. The results have implications for the design and analysis of future studies, and in the types of external data that can be used to augment analysis.

Stephen Edwards, Ph.D. Integrated Systems Toxicology Division, NHEERL, U.S. EPA, Research Triangle Park,

Biosketch: Dr. Stephen Edwards is a Systems Biologist within the U.S. Environmental Protection Agency's National Health and Environmental Effects Research Laboratory (EPA-NHEERL) in Research Triangle Park, N.C. Dr. Edwards is the EPA lead for an international effort to develop an Adverse Outcome Pathway (AOP) Knowledgebase, which is designed to house descriptions of the biological mechanisms underlying chemical toxicity in a structured manner. He is also leading an EPA effort to create computationally-predicted AOPs by integrating data from the published literature, omics databases, and HTS toxicity data. He serves as a senior advisor in the Office of Research and Development (ORD) on issues regarding the development of predictive toxicology models of disease using genomics, proteomics, and metabolomics. With a combination of experimental and computational experience, Dr. Edwards also serves as a liaison with the EPA's National Center for Computational Toxicology (NCCT) and has developed a flexible data management system to support systems biology research within the EPA. Dr. Edwards received his bachelor of science in chemistry from the University of North Carolina at Chapel Hill and his doctorate in pharmacology from Vanderbilt University Medical Center. Before joining the EPA, he served as a senior research scientist and research fellow at Rosetta Inpharmatics (Merck & Co.), in Seattle, Washington, a recognized leader in computational and systems approaches to drug development.

Abstract: AOPs & Biomarkers: Bridging High Throughput Screening and Regulatory Decision Making

As high throughput screening (HTS) approaches play a larger role in toxicity testing, computational toxicology has emerged as a critical component in interpreting the large volume of data produced. Computational models for this purpose are becoming increasingly more sophisticated requiring additional data sources to complement the HTS testing results. Biomarkers of effect can provide measurable data connecting the magnitude of perturbation from the in vitro system to a level of concern at the organism or population level. The adverse outcome pathway (AOP) concept provides an ideal framework for combining these two complementary data sources and providing a link between HTS results and the adverse outcomes that are typically used in regulatory decision making. An AOP Knowledgebase has been developed to organize and disseminate AOP-related information. In addition, systematic efforts to define AOPs and identify informative biomarkers for monitoring these AOPs in humans and other target organisms have resulted in several case studies showing the potential utility of this framework. [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.]

Platform Presentations:

T1

The Effect Of Changing Deoxyribonucleotide Concentrations On DNA Polymerase η Fidelity.

Beardslee RA, McCulloch SD. North Carolina State University, Raleigh, NC

DNA polymerase (pol) η is responsible for the bypass of both cyclobutane pyrimidine dimers (CPDs) and 8-oxoguanine (8-oxoG) during DNA replication. When copying DNA, error-prone pol η frequently misincorporates incorrect nucleotides contributing to mutagenesis and genomic instability. As relative dNTP concentrations can affect the rate of nucleotide misincorporation, we have investigated the role of changing dNTP concentrations on pol η 's error rate and hypothesized that nucleotide concentrations that approximate mammalian physiological ratios would alter certain rates of single base substitutions when copying both undamaged and damaged DNA. To study the effect of these mutations, we expressed the catalytic core of wild type human pol η in *E. coli*. Overexpressed protein was purified by chromatography using HiTrap™ Chelating HP (GE) with subsequent application of pol η rich fractions to Mono S™ (GE). Purified protein fractions and DNA oligomers were used in *in vitro* assays to evaluate DNA synthesis opposite templates with and without 8-oxoG and CPD lesions. Experiments show that the efficiency of primer elongation is limited when dNTP concentrations are unequal and that error rates and mutation spectrums observed are modified. Because dNTP concentrations vary throughout the cell cycle, we propose that these results suggest the fidelity of pol η is dynamic. Furthermore, as modification of dNTP levels is associated with cancer cells, cancer treatments, other environmental exposures as well as the DNA damage response we propose that this dynamic fidelity in concert with dysregulated polymerase levels could contribute to the mutator phenotype proposed to be significant to cancer progression.

NOTES:

T2

Molecular genetic analysis of mutation clusters formed in single-strand DNA

Kin Chan, National Institute for Environmental Health Sciences, Research Triangle Park, NC

Localized hypermutation (LHM) manifests as clusters of closely-spaced point mutations in human cancers. LHM can result from APOBEC cytidine deaminase activity on long stretches of single-strand DNA (ssDNA), suggesting that other mutagens can target ssDNA as well. We've devised a reporter system in budding yeast to characterize ssDNA-specific mutagens, which induce selectable mutation clusters only when ssDNA is generated (Figure 1).

We've validated this approach using two mutagens with known ssDNA-specificity: APOBEC3G and bisulfite. APOBEC3G-

induced uracils were excised to generate abasic sites, which were bypassed by error-prone translesion synthesis (TLS), creating equal proportions of C to T vs. C to G substitutions. In contrast, bisulfite induced a long-lived sulfonated uracil intermediate resistant to excision, whose bypass by TLS created mostly C to T transitions. Finally, we found that styrene oxide (a suspected environmental carcinogen) is an ssDNA-specific mutagen that induced mutation clusters where 53% of mutations originated at C, 26% at A, 18% at G, but none at T. Our results demonstrate the utility of this new approach to characterize poorly-understood environmental mutagens, including those that are apparently so weak that they can damage DNA significantly only in single-strand form.

NOTES:

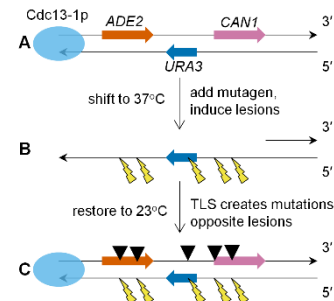


Figure 1: (A) Three reporter genes are embedded in subtelomeric region of *cdc13-1* haploid. (B) Shift to 37°C generates ssDNA, added mutagen induces lesions. (C) Return to 23°C, TLS creates mutations opposite lesions.

T3

Building gene expression signatures indicative of transcription factor activation to predict AOP modulation

VanDuyn, N¹; Franzosa, J²; Houck, K²; Ward, W²; Chorley, B²; Corton, C². ¹ORISE, RTP, NC, USA. ²NHEERL, U.S. EPA, RTP, NC, USA.

Adverse outcome pathways (AOPs) are a framework for predicting quantitative relationships between molecular initiating events (MIE) and downstream key events that lead to adverse outcomes. Defining gene sets (signatures) that predict chemical-induced MIEs (e.g., transcription factor (TF) activation) or key events (e.g., cell proliferation) would be useful in building models of AOP modulation that predict adverse outcomes. Current methods for identifying TF activation signatures have limitations. TF binding can be identified by experimental methods such as ChIP-Seq, but this is costly and time consuming, or by indirect measures such as in silico identification of TF binding sites, which may or may not be biologically relevant. Our approach is to identify the genes associated with TF activation via simultaneous assessment of TF activity and global gene expression in the same cell system (human hepatoblastoma cell line, HepG2). We have taken advantage of the Attagene FACTORIAL data (from ToxCast) and subjected the same RNA samples to microarray profiling of more than 47,000 RNA targets using the Illumina Human Expression BeadChip. Pearson's correlation and associated p-value were used to identify genes with a significant correlation between the change in expression and activation of AhR, TRalpha and PPARgamma across multiple chemical exposure experiments. The AhR signature was compared to a large human database of microarray experiments using the Running Fishers algorithm. These methods identified biosets associated with TCDD, benzo(a)pyrene and quercetin (p-value < 1×10^{-33}), confirming the utility of the method. This analysis can be extended to other TFs, allowing comprehensive assessment of chemical-induced modulation of human TFs in large, publically available, genomic datasets. (This abstract does not represent EPA policy.)

NOTES:

T4

Gas phase probe molecules for assessing *in vitro* metabolism to infer an *in vivo* response

Michelle M. Angrish¹, Michael C. Madden², and Joachim D. Pleil³. ¹ORISE Research Fellow, US EPA, Research Triangle Park, NC; ²Environmental Public Health Division, NHERL/ORD, US EPA, Chapel Hill, NC; ³Human Exposure and Atmospheric Sciences Division, NERL/ORD, US EPA, Research Triangle Park, NC

Efficient and accurate *in vitro* high-throughput screening (HTS) methods use cellular and molecular based adverse outcome pathways (AOPs) as central elements for exposure assessment and chemical prioritization. However, not all AOPs are based on systems biology, but rather supported by *in vitro* to *in vivo* extrapolation and other computational modeling. The challenge is to develop unambiguous quantitative links between *in vitro* responses and corresponding *in vivo* effects. The use of gas phase probe molecules (PrMs) supported by relevant human exposure studies and pharmacokinetic (PK) parameters may address this gap. Furthermore, existing HTS assays that require liquid handling robotics would be complemented by quantitative ultrasensitive gas phase PrM assays. We previously determined the kinetic parameters for methyl-tertiary butyl ether (MTBE) metabolism to tertiary butyl alcohol (TBA) via CYP2A6 and CYP2E1 pathways in the liver from human empirical data. In this study, we constructed a one-compartment PK model based on differential equations to estimate the MTBE probe pathway for establishing steady state *in vitro* liver function. Because the MTBE metabolic pathway is well characterized from *in vivo* data, we can use it as a PrM to explore chemicals effects on respective CYP pathways. We found that the PrM concept may provide a quantitative real time measurement from air of an *in vitro* response with a well-defined and corresponding *in vivo* effect. PrM methodology could be easily applied to a broad range of *in vitro* cell models and provides a novel approach to assess chemicals of concern including endocrine disruptors and volatiles.

NOTES:

Poster Presentations:

P1

Translesion Synthesis Error Induction in UV-B

Kimberly N. Herman¹, Scott D. McCulloch^{1,2}. ¹ Department of Biological Sciences, Environmental and Molecular Toxicology Program, ² Center for Human Health and the Environment, North Carolina State University, Raleigh, NC 27695

Ultraviolet light and many chemical agents are able to damage DNA. In normal systems this damage is usually able to be repaired, however in patients with xeroderma pigmentosum variant they are unable to bypass certain lesions due to a deficiency in active DNA polymerase η . In our studies we have evaluated both normal human fibroblasts (NHF) and xeroderma pigmentosum variant (XP-V) cells for mutations, cytotoxicity and mRNA expression changes caused by UV-B. We extended this study to also include analysis of mRNA expression in response to various chemical agents. In this study we used the HPRT assay to evaluate for the mutagenicity of UV-B in the presence and absence of pol η , as well as check for changes in mRNA expression after UV-B, cisplatin and MNNG. The mutation frequency is increased in the absence of pol η and the mutation spectrum is altered as well. Cytotoxicity of UV-B was only observed in the XP-V line in the presence of caffeine. There was also a concomitant rise in a substitute translesion polymerase is observed in the absence of pol η ; (Pol ι , Rev1, Rev3 depending on the treatment); for example after cisplatin treatment, there is a rise in DNA pol η in NHF cells but in XP-V cells the rise was in pol ι . This expression data along with mutation spectra demonstrates that mutations are caused by backup translesion synthesis polymerases whose fidelity for these lesions are lower than the preferred polymerase.

NOTES:

P2

Characterization of recombinant glutathione reductase from the psychrophilic Antarctic bacterium, *Colwellia psychrerythraea*.

Mikyoung Ji, Callie V. Barnwell, Amy M. Grunden. North Carolina State University, Raleigh, NC

Glutathione reductases catalyze the reduction of oxidized glutathione (glutathione disulfide, GSSG) using NADPH as substrate to produce reduced glutathione (GSH), which is an important antioxidant molecule that helps maintain the proper reducing environment of the cell. A recombinant form of glutathione reductase from *Colwellia psychrerythraea*, a marine psychrophilic bacterium, has been biochemically characterized to determine its molecular and enzymatic properties. *C. psychrerythraea* glutathione reductase was shown to be a homodimer with a molecular weight of 48.7 kDa using SDS PAGE, MALDI-TOF mass spectrometry and gel filtration. The *C. psychrerythraea* glutathione reductase sequence shows significant homology to that of *E. coli* glutathione reductase (66% identity), and it possesses the FAD and NADPH binding motifs, as well as absorption spectrum features which are characteristic of flavoenzymes such as glutathione reductase. The psychrophilic *C. psychrerythraea* glutathione reductase exhibits higher K_{cat} and K_{cat}/K_m at lower temperatures (4°C) compared to mesophilic Baker's yeast glutathione reductase. However, *C. psychrerythraea* glutathione reductase was able to complement an *E. coli* glutathione reductase deletion strain in oxidative stress growth assays, demonstrating the functionality of *C. psychrerythraea* glutathione reductase over a broad temperature range, which suggests its potential utility as an antioxidant enzyme in heterologous systems.

NOTES:

P3

A Rules Engine Approach to Supporting Adverse Outcome Pathway-based Rapid Toxicological Assessment

Kyle Painter¹, Ingrid L. Druwe¹, Erin E. Yost¹, Lyle D. Burgoon^{2, 1} Oak Ridge Institute for Science and Education / US Environmental Protection Agency, Research Triangle Park, NC, United States; ²National Center for Environmental Assessment, US Environmental Protection Agency, Research Triangle Park, NC, United States.

Despite gains in the efficiency of gathering data about chemical activity on biological assays, especially via High Throughput Screening (HTS) methods, there remains a large backlog of potentially harmful chemicals with little or no assessment of toxicity. Our project addresses that backlog in part by taking advantage of the recent development of Adverse Outcome Pathways (AOPs), and their collection in the AOP knowledge base. The part of the project described here is twofold: 1) scientists use AOP information to create a set of logical rules that define when knowledge of an upstream event(s) is sufficient to infer the existence of a downstream event. 2) A rules engine was built that can ingest data on a chemical derived from HTS assays and use the logical rules to infer other biological activity that results from that chemical. While the rules engine approach is not sufficient at this time to directly infer toxicity without a scientist's interpretation, this process will allow toxicologists to use HTS and other data to quickly screen chemicals and prioritize chemicals for further assessment, or to group chemicals together by disease/endpoint. This process may also be effective in illuminating data gaps and identifying where new assays may need to be developed or new data to be gathered to further aid toxicological assessment. The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the US Environmental Protection Agency.

NOTES:

P4

Predicting mutagenicity and carcinogenicity of Environmental Chemicals using Structure-Activity Relationship softwares

Arjun Keshava¹, Gopala Krishna². ¹Wake Early College of Health and Sciences, Raleigh, NC; ²Supernus Pharmaceuticals Research and Development, Rockville, MD.

Epinephrine, commonly known as adrenaline occurs naturally, but is used as a stimulant in cardiac arrest, anaphylactic shock and asthma attacks. Caffeine, also produced naturally, stimulates the central nervous system and provides a boost of energy. However, when consumed excessively causes headaches, dizziness, and abnormal heart rhythms. To understand the mutagenic and carcinogenic potential of caffeine and epinephrine, they were subjected to three commonly used structure-activity relationship (SAR) software. The three softwares used were DEREK, ToxTree, and VEGA. Publically available procedures and/or manuals were utilized to generate data outputs and the data was evaluated in comparison with available toxicological data on epinephrine and caffeine. The results of this study show that for epinephrine, no carcinogenicity or mutagenicity data output from either DEREK or ToxTree. The VEGA software predicted epinephrine to have possible carcinogenic effect, however not mutagenic. Similarly, there was no predictable output data for caffeine with either DEREK or ToxTree. Again, VEGA predicted no mutagenicity or carcinogenicity for caffeine. These results indicate that, based on the structure activity relationship software, neither compounds are carcinogenic or mutagenic with the exception of prediction of VEGA for epinephrine. It should be noted that additional toxicological studies are required to augment the prediction of SAR softwares.

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Mutagenicity-Emission Factors of Cookstoves: Correlations with PAH Emission Factors

S.H. Warren¹, E. Mutlu¹, S.M. Ebersviller¹, I.M. Kooter², J.A. Dye¹, J.J. Jetter¹, M.I. Gilmour¹, M. Higuchi¹, D.M. DeMarini¹ U.S. EPA, RTP, NC; ²TNO, Utrecht, The Netherlands

Emissions from solid fuels used for cooking cause ~4 million premature deaths per year. Introduction of advanced solid-fuel cookstoves is an interim solution to this problem, but such stoves should be assessed by appropriate performance attributes, including health effects. We evaluated two biomass cookstoves for one health-effects emission factor (mutagenicity) and 10 chemical-emission factors, correlated them, and compared the mutagenicity-emission factor to those of combustion emissions with known human-health effects. We burned red oak in a 3-stone fire (TSF), a natural-draft stove (NDS), and a forced-draft stove (FDS); we combusted propane as a liquid/gas control fuel. We determined emission factors based on the useful energy (megajoules_{delivered}) for carbon monoxide, nitrogen oxides (NOx), black carbon, methane, total hydrocarbons, 32 PAHs, PM2.5, levoglucosan (a wood-smoke marker), and mutagenicity in the *Salmonella* assay. Other than NOx all of the emission factors correlated highly among each other and were reduced on average 68 or 92% by the NDS or FDS, respectively, relative to the TSF. The mutagenicity-emission factor based on fuel energy (MJ_{thermal}) for the best stove (FDS) was similar to that of diesel exhaust, a human carcinogen. Mutagenicity- and many chemical-emission factors are equally informative for characterizing cookstove performance; however, appropriate emission factors are essential for characterizing the health effects of cookstoves. Under our conditions and by our analysis, a FDS is safer than a TSF, but it is still not safe to the primary user without adequate ventilation.

[Abstract does not necessarily reflect the views of the U.S. EPA.]

NOTES:

A framework for generating and leveraging computationally predicted AOPs

Shannon M. Bell^{1,2}, Stephen W. Edwards².¹ Oak Ridge Institute for Science and Education, ²Integrated Systems Toxicology Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711, USA

Given the vast number of chemicals with little health impact information, emphasis is placed on developing high throughput screening (HTS) methods for hazard prediction. Adverse Outcome Pathways (AOPs) represent an ideal framework for connecting molecular initiating events to key events, measured via HTS data, with adverse outcomes of regulatory importance. However, traditional AOP development is labor intensive and time consuming. We present a graph-based workflow, cpAOP-net, that enables data integration across multiple data types to identify computationally-predicted AOPs (cpAOPs). Case studies are presented illustrating use of cpAOP-net to generate a cpAOP for liver steatosis, identify targets for HTS, and compare cpAOPs for key events that might differentiate related outcomes. This framework for cpAOP identification has the potential to generate and rank hypothetical AOPs for expert evaluation and provide data-based scaffolds to improve the efficiency of AOP development. We discuss how cpAOP-net provides a mechanism for identifying HTS targets and the role of domain experts and confirmatory studies in model improvement. (The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency).

NOTES:

Reproducibility of Chemical Potency across Orthogonal *In Vitro* High Throughput Screening Assays

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Recent high throughput *in vitro* chemical screening (HTS) initiatives (e.g., ToxCast and Tox21) may be used to support future human and environmental risk assessments. Adverse outcome pathways (AOPs) will serve as a scaffold to achieve these goals. To support risk assessment using the AOP framework, we envision the use of orthogonal HTS assays, which are multiple assays that measure a molecular or cellular key event in different ways. Orthogonal assays provide weight of evidence for chemical perturbation of a key event within an AOP, and may be used to infer the associated dose. However, to be useful, the uncertainty associated with the potency measures by orthogonal assays must be understood. Here, a pilot study was conducted to examine the reproducibility of chemical potency across a suite of orthogonal assays at the same key event, focusing initially on chemicals that perturb the estrogen signaling pathway via binding and transactivation of the human estrogen receptor alpha (hER α). The NCBI PubChem database was used to identify potencies of 11 different chemicals in three orthogonal assays: 1) a radioligand binding assay that reports binding to the ligand binding domain (LBD) of the hER α ; 2) a cell-based assay (Invitrogen ER-alpha-UAS-bla GripTite™) that reports activation of an hER α LBD/Gal4 DNA binding domain fusion protein; and 3) a cell-based assay (BG1-Luc-4E2) that reports activation of endogenous full-length hER α . Variability in the reported potencies for each chemical was characterized by the residuals, with a mean calculated residual of -3.4×10^{-16} across chemicals. Bootstrap analysis indicated that, overall, potencies within a chemical were largely reproducible across assays, with a mean estimated residual of 5.8×10^{-3} (95% confidence interval from -1.6 to 2.1). Note that reproducible assays should have a mean estimated residual close to 0. While the sample size of this pilot was small, these data provide suggestive evidence that these orthogonal assays may be able to estimate a quantitative hazard level with relatively little uncertainty. We are expanding the scope of our work based on these results to include more chemicals and additional key events in other biological pathways. The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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Integrative data mining of high-throughput *in vitro* screens, and disease data to identify Adverse Outcome Pathway signatures.

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The Adverse Outcome Pathway (AOP) framework provides a systematic way to describe linkages between molecular and cellular processes and organism or population level effects. The current AOP assembly methods however, are inefficient. Our goal is to generate computationally-predicted AOPs (cpAOPs) via data mining to accelerate AOP assembly and provide a more comprehensive coverage of biological space. We used Frequent Itemset Mining (FIM) to find associations between the gene targets of ToxCast high-throughput screening (HTS) assays and disease phenotypes from the Comparative Toxicogenomics Database (CTD). ToxCast chemicals were used as aggregating variables for analyses. A cpAOP network was defined by considering genes and diseases as nodes and FIM associations as edges, thereby providing a graphical representation of the links and highlighting indirect associations. We illustrate an indirect association between AHR and glaucoma, suggesting a putative relationship. Though AHR isn't found in a CTD gene-disease query for glaucoma, it is a regulator of CYP1B1 (not screened in ToxCast) and the incorporation of additional datasets enabled detection of the putative relationship. This example highlights the value in integrating multiple data sources when defining cpAOPs for HTS data. (*The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA*).

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Bayesian Evidence Integration of Quantitative High Throughput Screening Data

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High Throughput Screening (HTS) assays have generated toxicological data on thousands of chemicals; however, it is unclear how this HTS data can be integrated to inform hazard identification and estimation of risk-specific concentrations/doses. In this pilot study, we examined how well Bayesian data integration methods may perform in integrating evidence from multiple HTS assay replicates. Specifically, we analyzed in vitro HTS data for a sample chemical, Bisphenol A (BPA), a potential xenoestrogen. HTS data for BPA (chemical ID: 6623), were acquired from the National Library of Medicine's PubChem Database for all active, confirmatory assays. Of the more than 1300 assays in PubChem, only 28 of these were confirmatory/quantitative assays. For this pilot study we selected confirmatory qHTS assays for estrogen receptor activation that tested a wide range of BPA concentrations. We used bootstrap meta-regression to identify the Point of Departure (POD) and the response/activity level at the POD, calculated the proportion of replicates with responses greater than the response/activity level at the POD, and used a flat prior distribution and a Beta distribution to model the likelihood, and thus, the posterior probability. In the future, we will use the posterior distribution to estimate the risk-specific concentration/dose at 1:1,000 and 1:10,000 risk. Bayesian analysis allows us to integrate evidence from multiple assays measuring estrogen receptor activation in a transparent manner. *(The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA. Mention of trade names or commercial products does not constitute endorsement or recommendations for use).*

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Establishing conditions for zinc-induced adaptive and adverse oxidative stress responses in human bronchial epithelial cells

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A key to understanding the adverse health effects associated with ambient air contaminants is determining molecular biomarkers that differentiate adaptive and adverse cellular processes. Zinc, a component of airborne particulate matter, presents an oxidant challenge to human lung. Here, we characterize the adaptive and adverse cellular responses of a normal human bronchial epithelial cell line (BEAS-2B) to zinc exposure *in vitro*. Cytotoxicity and markers of oxidative stress and apoptosis were assessed in BEAS-2B cells exposed to Zn²⁺ with 1 μM pyrithione, which facilitates cellular uptake. After 48h, markedly reduced viability was observed in cells treated with 3 μM Zn²⁺ (31±5%), but not cells exposed to 1 μM Zn²⁺ (104±4%) when compared with unexposed cells. Nuclear protein of the antioxidant stress factor, NRF2, increased 2.6-fold in cells exposed to 3 μM Zn²⁺ after 2 and 4 hours. Moreover, apoptotic markers Bak and Lamin B levels increased in these cells from 38 and 25 ng/mL after 24 h to 67 and 30 ng/mL after 40 h, but decreased to 49 and 26 ng/mL after 48 h. Visual inspection revealed that a portion of the cells treated with 3 μM Zn²⁺ appeared to recover as evidenced by returning to a flattened, attached morphology between 40 and 48 h. These data suggest that the switch between adaptation and apoptosis in our model begins to occur at exposures of approximately 3 μM Zn²⁺ and as early as 24h after exposure. *This abstract does not necessarily reflect the policy of the US EPA.*

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Scientific text extraction using FIDDLE: a foundation for accurate literature mining.

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A principal aim of the ToxCast high-throughput screening program is to increase the efficiency with which potentially dangerous chemicals can be evaluated with regard to their impact on human health. With similar intent, recent research has demonstrated the usefulness of text-mining and natural language processing to extract valuable information about the likely biological impact of chemicals from latent information and relationships hidden in the scientific literature. In practice, however, an important technical obstacle to the wider applicability of these methods has been the difficulty of accurately extracting chemical names and other scientifically relevant text from a large corpus of scientific documents. This is difficult not only due to the fact that chemical nomenclature is so variable and imprecise, but also because the portable document format (PDF), in which most scientific texts are published, sacrifices word order and natural text flow in favor of a more graphically-oriented representation. Here we introduce the ‘Flow-Intelligent Document Decoder for Literature Extraction’ (FIDDLE), a novel algorithm and a tool under development, which can accurately extract text from PDF documents. In addition to preserving the correct word order across columns, figures and tables, FIDDLE is also able to accurately sectionalize scientific documents in an automated fashion. When key scientific terms (e.g. chemical names) are mentioned in different document sections (e.g. title, abstract, methods, references, etc.) they may carry greatly different implications. Therefore, the ability to correctly divide documents into meaningful sections is a critical capability in the context of literature mining. When combined with a custom built, dictionary-based chemical name recognizer, FIDDLE is able to accurately extract ToxCast/Tox21 chemical names from full text PubMed manuscripts at a rate several times greater than would otherwise be possible using only the associated MeSH terminology or the text of the titles and abstracts. Our approach offers the ability to perform full text extraction of scientifically relevant keywords (e.g. chemical name or phenotypic endpoint) and in turn greatly enriches text and literature mining capabilities with wide ranging applicability in the environmental and health sciences.

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