4th Annual Meeting
of the
American Society for Cellular and Computational Toxicology

Integrated Approaches to Testing and Assessment: Promises and Challenges of a More Flexible Approach to Toxicology Testing

October 1-2, 2015
Environmental Protection Agency
Durham, NC
Welcome everyone to the 4th annual meeting of the American Society for Cellular and Computational Toxicology! If you’re reading this, our Congress has managed to avoid yet another shutdown of our Federal government!?! Thanks to the US EPA for hosting us at their wonderful facility in Research Triangle Park, North Carolina. We’re continuing to build momentum as our membership continues to grow with the ever increasing interest in cellular and computational approaches to toxicology testing, and what better place to come together than the home of EPA’s National Center for Computational Toxicology.

Once again, the organizing committee has pulled together an exceptional program for you to enjoy over the next day and half. We’re focusing on integrated approaches to testing and assessment, and how combining in vitro and in silico approaches can be used in regulatory and investigational toxicology. We have a distinguished group of invited speakers who will be addressing various aspects of these approaches, and I invite all attendees to interact with our speakers as much as possible – not only during the planned discussion session, but during the multiple breaks included in the program. I know you’ll also enjoy hearing from those selected by the Organizing Committee for a platform presentation based on their outstanding abstract submissions.

Many of you have also expended the time and energy to share your research efforts by preparing posters. I want to encourage all attendees to show their appreciation by taking advantage of the poster discussion session on Thursday afternoon. Interactions at these poster sessions should be an integral part of attending this annual meeting. We’ll also be recognizing the inaugural recipient of the Edward Carney Predictive Toxicology Award, an annual award that will be given to an outstanding poster presentation in memory of our former colleague who was a true pioneer in our field.

Of course, there is a long list of people that have contributed to making this meeting happen, but let me make sure to give all the credit to the annual meeting organizing committee who have put together this fine program. Please thank Jack Fowle, Marilyn Aardema, Erin Hill, Jie Shen, Natalia Vinas, and Kristie Sullivan for putting forward their time and effort in designing the program and contacting the speakers. Thanks also to ASCCT member Tom Knudsen for serving as our host to provide us access to this great facility. And please make sure to once again commend Kristie for her continued oversight of an outstanding webinar program that we’ve all enjoyed over the past year.

Finally, I want to thank - and congratulate - each of you for becoming ASCCT members. Many of you have convinced your company management to be financially supportive as well, and I’d like to encourage others to do the same. We continue to operate on a relatively thin margin, and there are many more things we can do to benefit our members if our finances allowed. Speaking of support, I should now take the time to thank the organizations whose contributions have made much of this annual meeting possible – Alternatives Research and Development Foundation,
Center for Alternatives to Animal Testing, Institute for In Vitro Sciences, Integrated Laboratory Systems, Inc., Physician’s Committee for Responsible Medicine, and PETA International Science Consortium, Ltd.

And don’t forget, the ASCCT was originally envisioned as a platform where regulatory and research scientists from both the computational and cellular sides of toxicology could freely exchange ideas, so please make it happen!

Your president,
Dr. David Allen

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Please join us in thanking our past board members for their service, and in welcoming our new members, included in the list below.

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Integrated Approaches to Testing and Assessment: Promises and Challenges of a More Flexible Approach to Toxicology Testing

4th Annual ASCCT Meeting
October 1-2, 2015
Environmental Protection Agency
109 T W Alexander Drive C261, Durham, NC 27705

Thursday October 1

9:00-9:15: Welcome and Introduction: David Allen, Integrated Laboratory Systems, Inc. & ASCCT President

9:15-10:00: Moving Beyond One Test: Leveraging the Whole Toolbox for Integrated Decision Strategies, Warren Casey, NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

10:00-11:30: IATA Application Case Studies

1. Developing non-testing IATA informed by mechanistic insights: Case studies in building scientific confidence, Grace Patlewicz, NCCT, Environmental Protection Agency
2. OECD implementation of IATA demonstrated by the skin sensitization endpoint, Johanna Matheson, Consumer Product Safety Commission
3. Why and how do we incorporate metabolism and kinetics in integrated testing and assessment, Miyoung Yoon, The Hamner Institutes for Health Sciences

11:30-1:30: Poster Viewing and Lunch [author attended 11:30-12:30]

1:30-2:00: Use of High Throughput Assays and Predictive Models in the U.S. EPA Endocrine Disruptor Screening Program, David Dix, OSCP, EPA

2:00-3:45: Selected Abstracts

2:00-2:20: Harmonization of the Regulatory Use of Non-animal Methods in Chemical Testing through the increased use of Integrated Approaches to Testing and Assessment, Catherine Willett, The Humane Society of the United States

2:20-2:40: Machine Learning Approaches for Predicting Human Skin Sensitization Hazard, Judy Strickland, ILS, Inc./NICEATM

2:40-3:00: Transforming In Vitro Susceptibility Modeling: Integrating Cellular Signaling, Epigenetics, and Inter-individual Variability within the Human Airway, Shaun D. McCullough, EPHD, EPA
3:00-3:20: Computational Modeling of Male Reproductive Tract Development for Use in Predictive Toxicology, Maxwell C. Leung, NCCT, EPA

3:20-3:40: Use of Adverse Outcome Pathways in the Design of Integrated Approaches to Testing and Assessment: A Case Study of Pulmonary Fibrosis Induced by Carbon-Based Nanomaterials, Ashley DeCoux, PETA International Science Consortium

3:40-4:00: Break

4:00-4:30: ASCCT Annual Business Meeting

4:30-6:00: Reception and Poster Viewing

Friday October 2

9:00-9:45: Chemicals and Risk: New Approaches to Current Practices, Craig Rowlands, Dow Chemical Company

9:45-12:00: Selected Abstracts

9:45-10:05: Development and Application of Liver Bioreactor to Streamline Metabolite Identification and Toxicity Testing, J.M. Pedersen, The Hamner Institutes for Health Sciences

10:05-10:25: Product registration of antimicrobial cleaning products and other pesticides using updated US EPA nonanimal testing strategy for eye irritation, Amy Clippinger, PISC

10:25-10:45: Monitoring Bioenergetic Effects of Environmental Quinones in Human Airway Epithelial Cells Using Extracellular Flux Technology, Katelyn S. Lavrich, UNC Chapel Hill

10:45-11:15: Break

11:15-11:35: Computational approach that predicts genotoxic mode of action based on data from a multiplexed gH2AX- and phospho-H3-based flow cytometric assay, Steven M. Bryce, Litron Laboratories


12:00-1:00: Panel Discussion

Warren Casey, NICEATM
Miyoung Yoon, The Hamner
Craig Rowlands, Dow Chemical
Joanna Matheson, CPSC (invited)
Grace Patlewicz, EPA
Invited Speaker Abstracts
Moving Beyond One Test: Leveraging the Whole Toolbox for Integrated Decision Strategies

Warren Casey

NTP Interagency Center for the Evaluation of Alternative Toxicological Methods

To date, much of the effort to develop non-animal alternative testing methods has focused on 1:1 replacement; the replacement of one animal-based test with a single in vitro assay. Given the complexity of mammalian physiology, it is not surprising that this approach has achieved limited success. Advances in the diverse fields of science, from molecular biology to biomedical engineering to computational toxicology, have provided new tools that provide the potential to understand physiological processes on a level previously unachievable. However, we must learn how to best combine both information and insight from these diverse fields if we hope to capitalize on the advances made in each. Exploring methodologies to integrate technologies and data streams is extremely important, but the more difficult task of collaborating with colleagues in diverse disciplines is vital if we hope to achieve the noble goal of replacing the use of animals in biomedical research and toxicity testing. This talk will provide an overview of advances made, challenges encountered, and possible future directions for using integrated approaches for understanding complex physiological processes.
Developing Non-testing IATA Informed by Mechanistic Insights: Case Studies in Building Scientific Confidence

Grace Patlewicz
U.S. Environmental Protection Agency

Non-testing approaches encompassing (Q)SARs, chemical categories and read-across have enjoyed a revival in recent years following changes in the global regulatory landscape. Whilst the uptake of these non-testing approaches for regulatory purposes is very encouraging, their practical application suffers from certain shortcomings. Some regulatory endpoints lend themselves to robust (Q)SAR development, whilst others such as repeated dose toxicity are too complex to be modelled in this way and despite the role that read-across can play, acceptance is still in part thwarted by the difficulty in addressing uncertainties. Adverse Outcome Pathways (AOPs) which are useful constructs for representing existing knowledge concerning the causal linkages between a molecular initiating event and an adverse outcome could offer some practical solutions in addressing at least some of these difficulties. AOPs themselves provide the mechanistic basis for generating, integrating and interpret non-standard information for key events (KEs) in a manner that can be applied in decision making. The framework for this is Integrated Approaches to Testing and Assessment (IATA). Indeed an IATA focused on existing data and other non-testing approaches marks a significant change in how read-across and (Q)SAR approaches could be developed and applied in the future. Instead of predicting the adverse outcome, future (Q)SARs could be developed to model individual KEs – thus providing some information on the chemical applicability domain. Uptake and acceptance of AOP-informed IATA and their associated elements will still necessitate some level of validation to demonstrate scientific confidence for specific purposes. Here we describe a scientific confidence framework for Tox21 approaches anchored in AOPs and illustrate how this can be used to direct the development of non-testing approaches and their application as part of IATA. Case study examples will illustrate insights derived from various endpoints that are informed by mechanistic information including that arising from AOPs. The views expressed are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.
**Why and How Do We Incorporate Metabolism and Kinetics in Integrated Testing and Assessment**

**M. Yoon, J. Pedersen, Y. Zhao, M. E. Andersen, and H. J. Clewell**

The Hamner Institutes for Health Sciences

To support the global paradigm shift in toxicology toward the use of *in vitro* approaches for assessing chemical risk and safety, development of new tools and improvement of previously available tools in experimental and computational modeling approaches are underway in a number of global research initiatives. Integrated testing strategies are required to coherently combine key information obtained from these multiple *in vitro* and *in silico* tools with specific purposes; cell-based toxicity assays for prediction of human biological effects, computational systems pathway modeling to define safety levels *in vitro*, and biokinetic and *in vitro* to *in vivo* extrapolation (IVIVE) modeling to translate *in vitro* findings in the context of *in vivo* human safety. This talk will describe the need to incorporate metabolism and kinetics in integrated testing strategies as a key to the successful extrapolation of *in vitro* conditions to comparable in-life conditions. The emphasis will be on describing the development of improved *in vitro* metabolism tools and advanced biokinetic modeling to support accurate dose extrapolation between *in vitro* and *in vivo* scenarios. To this end, a liver bioreactor and modified Caco-2 cell model will be described with case study examples including 7-ethoxycoumarin, acetaminophen and parabens. Also, application strategies of these *in vitro* experimental models to address the major obstacles for *in vitro*-based prediction of human safety, namely prediction of metabolite-mediated toxicity and prediction of repeated exposure toxicity for systemic effects, will be discussed (supported by ACC-LRI).
Use of High Throughput Assays and Predictive Models in the U.S. EPA Endocrine Disruptor Screening Program

David J. Dix

U.S. Environmental Protection Agency

The U.S. Environmental Protection Agency’s (EPA) Endocrine Disruptor Screening Program (EDSP) is incorporating an alternative scientific approach to screen chemicals for their ability to interact with the endocrine system. This shift will improve the Agency’s ability to fulfill its statutory mandate to screen pesticide chemicals and other substances for their ability to cause adverse effects by their interaction with the endocrine system. The approach incorporates validated high throughput assays and a predictive model and, based on current research, can serve as an alternative for some of the current assays in the Endocrine Disruptor Screening Program (EDSP) Tier 1 battery. EPA has partial screening results for over 1800 chemicals that have been evaluated using high throughput assays and a predictive model for the estrogen receptor pathway. In the future, EPA anticipates that additional alternative methods will be available for EDSP chemical screening based on further advancements of high throughput assays and predictive models for other endocrine pathways. Use of these alternative methods will accelerate the pace of screening, decrease costs, and reduce animal testing. In addition, this approach advances the goal of providing sensitive, specific, quantitative, and efficient screening using alternative test methods in the Tier 1 battery to protect human health and the environment. The application of these alternative, innovative tools for screening chemicals for endocrine bioactivity represents the first step in a paradigm shift for chemical safety testing, and the first systematic application of EPA’s ToxCast data in an EPA regulatory program.
**Chemicals and Risk: New Approaches to Current Practices**

J. Craig Rowlands

The Dow Chemical Company

Increased societal demands for more sustainable chemicals includes reducing their inherent hazards, which in turn is driving improvements in the design criteria for new chemicals. Practical challenges for implementing design criteria for alternative replacement chemicals must also factor in adequate chemical performance and economics compared to the chemicals considered for replacement. The new tools for predictive toxicology are well suited for assisting with the molecular design of sustainable chemicals and the information generated from predictive toxicology assays. Predictive toxicology methods are also considered for Integrated Approaches to Testing and Assessment (IATA) to develop more intelligent testing programs, thereby reducing the use of animals by eliminating unsuitable candidate chemicals early and supporting the safety of promising candidates.
Harmonization of the Regulatory Use of Non-animal Methods in Chemical Testing Through the Increased use of Integrated Approaches to Testing and Assessment

Catherine Willett, Ph.D.

The Humane Society of the United States, 2100 L Street NW, Washington, DC

There are scientific, economic, practical and regulatory pressures stimulating the development and use of streamlined chemical testing and assessment approaches, including an increased reliance on non-animal methods. Advances in biological understanding as well as experimental technologies (e.g. ‘omics tools, stem cell culturing, reconstructed tissues), have allowed the consideration of dramatically different approaches to understanding disease and toxicology than those traditionally practiced, and increasingly, collections or batteries of non-testing and non-animal test methods are starting to replace apical animal tests. Articulation of the underlying perturbations of biology, for example in the form of adverse outcome pathways (AOPs) that link molecular initiating events to key intermediate events to the adverse outcome, can be used to form the logical basis for the integration of tests that query key events in a pathway in order to develop appropriate integrated approaches to testing and assessment (IATA). It is anticipated that these testing strategies can improve the efficiency of hazard assessment, and, within the context of exposure and specific regulatory applications, risk assessment. In light of number and variety of new methods and types of information that are replacing traditional tests, new approaches are needed to support both assay validation and harmonized regulatory use. The Organization for Economic Cooperation and Development (OECD) Environment Directorate has made a significant investment in development of AOPs, and is currently developing guidance for IATA. OECD is also developing guidance to streamline validation of these novel technologies. This combined guidance, in the form of IATA supported by AOPs, along with streamlined assay validation, has the potential to facilitate the harmonized use of novel and varied methodologies. This talk will discuss how the OECD guidance along with other considerations could facilitate harmonized acceptance of new, particularly in vitro, assessment methods.
Machine Learning Approaches for Predicting Human Skin Sensitization Hazard

J. Strickland¹, Q. Zang¹, M. Paris¹, N. Kleinstreuer¹, D.M. Lehmann², D. Allen³, J. Matheson³, A. Jacobs⁴, A. Lowit⁵, W. Casey⁶

¹ILS/NICEATM, RTP, NC, USA; ²EPA/ORD/NHEERL, RTP, NC, USA; ³CPSC, Rockville, MD, USA; ⁴FDA/CDER, Silver Spring, MD, USA; ⁵EPA/OCSPP/OPP, Washington, DC, USA; ⁶NIH/NIEHS/DNTP/NICEATM, RTP, NC, USA

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One of ICCVAM's top priorities is the development and evaluation of non-animal approaches to identify potential skin sensitizers. The complexity of biological events necessary for a substance to elicit a skin sensitization reaction suggests that no single in chemico, in vitro, or in silico method will provide a complete replacement for currently accepted animal tests. Thus, ICCVAM is evaluating machine learning approaches to integrate relevant data based on the OECD adverse outcome pathway for skin sensitization to predict human skin sensitization hazard. Models were built and tested using input variables derived from public data for 96 chemicals with human skin sensitization hazard information. Data were obtained from three in chemico or in vitro methods (direct peptide reactivity assay [DPRA], human cell line activation test [h-CLAT], and KeratinoSens assay) and six physicochemical properties (octanol: water partition coefficient [log P], water solubility, vapor pressure, molecular weight, melting point, and boiling point). A read-across prediction of skin sensitization hazard for each substance, produced using OECD QSAR Toolbox, provided an additional input. All of these data were used as potential features to predict human hazard using two machine learning approaches, support vector machine and logistic regression, applied to 12 combinations of features. Models were trained on a set of 72 substances and tested on an external set of 24 substances. The feature set containing DPRA, h-CLAT, KeratinoSens, OECD QSAR Toolbox, and log P produced the best performing model by either approach: accuracy = 99% (71/72), sensitivity = 98% (50/51), and specificity = 100% (21/21) for the training set; and accuracy = 96% (23/24), sensitivity = 93% (14/15), and specificity = 100% (9/9) for the test set. The performance of this integrated approach was better at predicting human skin sensitization hazard than the local lymph node assay or any of the in chemico, in vitro, or in silico methods alone. These data suggest that computational methods are promising tools to effectively identify potential skin sensitizers without testing in animals. This project was funded in whole or in part with Federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.
Transforming In Vitro Susceptibility Modeling: Integrating Cellular Signaling, Epigenetics, and Inter-individual Variability Within the Human Airway

Shaun D. McCullough1, Emma C. Bowers1,2, Doan M. On1, David S. Morgan1, Lisa A. Dailey1, David Diaz-Sanchez1, and Robert B. Devlin1

1Environmental Public Health Division, U.S. Environmental Protection Agency, Chapel Hill, NC 27599
2Curriculum in Toxicology, University of North Carolina at Chapel Hill, Chapel Hill, 27599
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Epigenetic regulators, such as chromatin modifications and DNA methylation, function as critical and dynamic mediators of gene expression and shape the way cells, tissues, and individuals respond to their environment. Despite the advancement of many model systems, relatively little has been done to examine the role of the epigenome in exposure effects and susceptibility in vitro. Air pollutant exposure is associated with increased cardiopulmonary morbidity and mortality; however, there is a broad range of responsiveness between individuals that is not well explained by current susceptibility models and the mechanisms underlying this inter-individual variability remain elusive. As the barrier between the lung and the environment, the airway epithelium plays a critical role as a modulator of pro-inflammatory and oxidative stress in response to environmental exposures. By taking advantage of air-liquid interface culture with primary airway epithelial cells from a panel of donors, we have examined the relationship between cellular signaling networks, baseline epigenetic states, and inter-individual variability of pro-inflammatory responsiveness to the model air pollutant ozone. During the course of this study, we identified two distinct mitogen activated protein (MAP) kinase pathways (EGFR/MEK/ERK and MKK4/p38) as the drivers of the cellular response to ozone, which varies from traditionally accepted findings in cell lines implicating the NF-κB pathway. While the ozone-mediated induction of pro-inflammatory cytokines (IL-8, IL-6, COX2, IL-1α, and IL-1β) varied between donors, the relative activation of MAPK signaling was similar. Given this similarity, we hypothesized that the variability in responsiveness originated downstream of MAPK signaling, specifically the patterns of chromatin modification within the regulatory regions of target pro-inflammatory genes. We identified pre-exposure patterns of chromatin modifications (histone H3 lysine 27 acetylation and lysine 4 methylation) that correlated with the magnitude of post-exposure pro-inflammatory gene expression. The findings from this study highlight the utility of advanced in vitro models in modern mechanistic and epigenetic toxicology. They also contribute to the establishment of physiologically relevant in vitro models as the foundation of the emerging field of epigenetic toxicology, which will ultimately play a critical role in our understanding of exposure associated health effects and susceptibility.
Computational Modeling of Male Reproductive Tract Development for Use in Predictive Toxicology

Maxwell C. Leung 1,2, Thomas B. Knudsen3

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The genital tubercle (GT) is a rudimentary embryological structure that diversifies into male or female phenotypes, depending on the fetal hormonal environment. Prior to sexual differentiation, GT formation is controlled by signaling pathways from endodermal (e.g., SHH) and mesenchymal (FGF) cells resulting in polarized outgrowth and urethral plate extension. Subsequent phenotypic differentiation is conditioned by androgen production from the fetal testis, leading to enhanced cell growth in the preputial mesenchyme and urethral tube closure in males. Absence of androgen defaults the GT to the female phenotype, and partial disruption by anti-androgenic compounds (e.g., vinclozolin) can result in microphallus or urethral closure defects (hypospadias). To model the complex interactions between morphoregulatory, endocrine, and environmental influences on GT development, we built a multicellular computer model of the GT that simulated GT androgenization. The model was constructed in CompuCell3D and implemented spatially dynamic signals (e.g., SHH, FGF10, and androgen) that modulated stochastic cell behaviors (e.g., differential adhesion, cell motility, proliferation, and apoptosis). Several cell types were anatomically represented in the model and programmed to direct GT outgrowth and urethral closure when given an androgen stimulus. The emergent property of urethral closure was found to be dependent on ventral mesenchymal proliferation and urethral plate endodermal apoptosis under control of androgen receptor (AR) signaling. These cell-agent based models: 1) provide a platform for integrating available biological information to predictively model the complex pathogenesis of male reproductive tract development; 2) enable the generation of new research hypotheses and ‘what-if’ scenarios; and 3) contribute to better mechanistic understanding of key events underlying adverse development of a complex embryological system. Most importantly, these models produce probabilistic predictions of urethral closure defects from chemical effects on SHH, FGF10, and androgen simulations. This abstract does not necessarily reflect US EPA policy.
Use of Adverse Outcome Pathways in the Design of Integrated Approaches to Testing and Assessment: A Case Study of Pulmonary Fibrosis Induced by Carbon-Based Nanomaterials

Ashley DeCoux¹, Jake Nikota², Sabina Halappanavar², Amy Clippinger¹

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An adverse outcome pathway (AOP) is a conceptual framework that uses existing mechanistic knowledge concerning a chemical’s mode-of-action in order to identify a molecular initiating event (MIE) and key events (KEs) that lead to an adverse outcome (AO). AOPs provide a means of systematically organizing existing scientific information, identifying knowledge gaps, informing the design of integrated approaches to testing and assessment (IATAs), and guiding research priorities. The AOP Wiki, which is an interactive and virtual encyclopedia for AOP development, has been created by the European Commission’s Joint Research Centre, the U.S Environmental Protection Agency, and the Organisation for Economic Co-operation and Development (OECD). An AOP for skin sensitization initiated by covalent binding to proteins has been published by the OECD, and other AOPs are under development. Recently, a putative AOP for pulmonary fibrosis was accepted by the OECD for further development.

Due to their unique mechanical, thermal, and electrical properties, multi-walled carbon nanotubes (MWCNTs) are extensively used in industrial and consumer applications, thereby increasing exposure and necessitating the development of approaches for toxicity assessment. The most extensively reported AO for exposure to MWCNTs is the development of pulmonary fibrosis. Pulmonary fibrosis is an interstitial lung disease that is associated with the inhalation of substances, including MWCNTs. Because complex molecular mechanisms are involved in the development of fibrosis, a single in vitro assay that can predict in vivo fibrosis has not been developed. An IATA capable of accurately assessing different KEs involved in the fibrotic response is required.

In an AOP case study, existing mechanistic information concerning MWCNTs and pulmonary fibrosis was organized, and the MIE and KEs involved in the process were identified. This information was then used to inform the design of pertinent in vitro assays for prediction of lung fibrosis in humans. Specifically, biomarkers of KEs, such as the expression of pro-inflammatory cytokines, ROS generation, fibroblast proliferation, and collagen accumulation, can be measured in lung cells cultured at the air-liquid interface to predict the development of pulmonary fibrosis in humans. This example shows how AOPs can be used to streamline testing and to design IATAs.
Development and Application of Liver Bioreactor to Streamline Metabolite Identification and Toxicity Testing


The Hamner Institutes for Health Sciences, Research Triangle Park, NC

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Estimation of human kinetics, especially metabolism, from in vitro data and computational modeling is a key component in integrated testing approaches for non-animal based safety assessments. Despite recent advances in hepatocyte culture techniques, the major challenge is predicting in vivo metabolite profiles from in vitro data. The aim of this project was to develop a hepatic in vitro model that generates in vivo-relevant metabolite profiles in sufficient amounts for rapid metabolite identification.

To this end, primary hepatocytes were cultured for 30 days as 3D cultures in hepatocyte-alginate beads with high cell-to-media ratio, a key factor to obtain the desired yield of metabolites. Dynamic culture conditions were applied and 3D fluid dynamic modeling was conducted to resolve optimal encapsulation and culture conditions in the chamber. Metabolic competence was measured weekly using well characterized compounds including 7-ethoxycoumarin (7-EC) and acetaminophen (APAP).

The results show that alginate bead encapsulated hepatocytes are able to sustain metabolic capacity at a level comparable to freshly isolated cells for the first seven days in culture for both 7-EC and APAP. A reduced metabolic activity was observed after two weeks in culture. However, if cultured in the flow based Quasi-vivo® culture system metabolic capacity was regained. Planned future experiments include incubations of chemicals with high human exposure concerns to identify potentially active metabolites that can be expected in vivo. In conclusion, the developed model can provide key metabolism support for non-animal based toxicity testing. In combination with in vitro to in vivo extrapolation and PBPK modeling with reverse dosimetry it can be used to estimate relevant human parent and metabolite exposure (ACC-LRI funding is gratefully acknowledged).
Product Registration of Antimicrobial Cleaning Products and Other Pesticides using Updated US EPA Nonanimal Testing Strategy for Eye Irritation

Amy Clippinger1, Patricia Bishop1, Gertrude-Emilia Costin2, Erin Hill2, Hans Raabe2

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A joint effort between several antimicrobial cleaning product (AMCP) companies and the US Environmental Protection Agency (EPA) has led to the development of a decision tree approach using three in vitro/ex vivo assays to determine eye irritation under the EPA Office of Pesticide Programs’ (OPP) hazard classification and labeling system.

Starting in 2004, EPA, seven consumer product companies, the Institute for In Vitro Sciences (IIVS), and the Accord Group collaboratively initiated a comparison of historic in vivo Draize rabbit eye irritation data with data from one or more of three alternative methods: the ex vivo Bovine Corneal Opacity and Permeability (BCOP) assay, the in vitro EpiOcular (EO) assay, or the Cytosensor Microphysiometer (CM) assay.

The National Toxicology Program’s Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) conducted a technical review of the submitted data to determine the effectiveness of the alternative methods in predicting OPP hazard labeling categories for eye irritation of AMCPs. In a subsequent 18-month pilot program, the EPA encouraged companies to submit in vitro data or parallel sets of in vivo and in vitro data in order to further evaluate and gain experience with the alternative eye irritation methods. The results showed that the BCOP, EO, and CM tests could be used either singly or in combination to differentiate among the four eye irritation hazard categories currently used by OPP. A decision tree approach is employed for selecting the appropriate assay(s) based on product chemistry and water solubility.

The EPA issued a policy document in 2013 that allowed for an alternative testing framework using these methods when classifying eye irritation potential of AMCPs. In 2015, the policy was updated to expand the use of the BCOP for identifying category III eye irritants. Importantly, this testing strategy has been successfully applied to support the registration of new AMCPs. In addition to the testing of AMCPs, the EPA will consider the use of this testing strategy for the evaluation of pesticide products other than AMCPs on a case-by-case basis.
Monitoring Bioenergetic Effects of Environmental Quinones in Human Airway Epithelial Cells using Extracellular Flux Technology

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Numerous human health effects of ambient air pollution are attributed to oxidative stress, though the exact mechanisms are poorly understood. Quinones are ubiquitous components of ambient air pollution, released into the environment through fuel combustion and tobacco smoke. Those detected most abundantly in ambient air include 1,2-naphthoquinone (1,2-NQ), 1,4-naphthoquinone (1,4-NQ), 9,10-phenanthrenequinone (9,10-PQ), and 1,4-benzoquinone (BQ). Quinones vary in their ability to catalyze redox cycling to produce reactive oxygen species and bind proteins and DNA electrophilically. Exposure of human airway epithelial cells to 1,2-NQ has been shown to induce adaptive gene signaling pathways. Mitochondria are key regulators of oxidative stress and are linked to these signaling pathways. The recently developed Seahorse Biosciences XF Analyzer uses fluorogenic probes to simultaneously measure oxygen consumption rate (OCR) and extracellular acidification rate (ECAR), markers of mitochondrial function and glycolysis respectively. We sought to better define the effect of environmentally relevant quinones on mitochondrial function and other bioenergetics parameters using extracellular flux technology in human airway epithelial cells. The redox-cycling quinones, 1,2-NQ, 1,4-NQ, and 9,10-PQ, significantly increased OCR, reaching peak effect within five minutes. This OCR rise is still seen when cells are treated first with the mitochondrial inhibitor rotenone then exposed to 1,2-NQ, indicating that the effect is independent of mitochondrial electron transfer. Two hour glucose starvation, which reduces cellular NADPH levels, strongly blunted this 1,2-NQ induced OCR rise and is evidence that this effect is largely due to redox cycling of the quinone. BQ, which does not redox cycle, does not affect OCR. Preliminary assays in isolated mitochondrial systems suggest that 1,2-NQ inhibits mitochondrial function. The opposing effects seen in cellular and isolated mitochondrial systems highlight the importance of understanding cellular processes of a compound in toxicological assessment using extracellular flux technology. This abstract of a proposed presentation does not necessarily reflect EPA policy.
Computational Approach that Predicts Genotoxic Mode of Action Based on Data from a Multiplexed γH2AX- and Phospho-H3-based Flow Cytometric Assay

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Candidate phenotypic signatures of clastogenicity, aneugenicity, and cytotoxicity were multiplexed into a single add-and-read-type flow cytometric assay. Reagents included a proprietary combination of detergents to liberate nuclei, propidium iodide and RNase to serve as a pan-DNA dye, fluorescent antibodies against γH2AX, cleaved Parp, and phospho-H3 (p-H3), and Counting Beads for absolute nuclei counts. TK6 cells were exposed to each of 69 chemicals in 96 well plates. Exposure to 20 concentrations was continuous for up to 24 hrs, and the highest concentration was generally 1 mM. At 4 and 24 hrs, aliquots were removed and added to microtiter plates containing the reagent mix. Robotic sampling facilitated walk-away data acquisition. For logistic regression modeling purposes, we reduced the data by selecting one equitoxic concentration for each chemical (i.e., relative nuclei count of approximately 20%). The strongest model was: 4hr γH2AX and p-H3 values, and 24 hr polyploidy values. This three-factor model resulted in 91% concordance with our a priori chemical classification calls. A leave-one-out validation exercise was performed, and in this case 87% concordance was observed. The misclassified chemicals were aneugens with potent protein kinase inhibition profiles, and clastogens that primarily affect non-DNA targets. These initial results are encouraging, as they suggest that a rapid, multiplexed technique may be capable of providing genotoxic mode of action information with remarkable efficiencies. More work is planned with a broader range of chemicals, other cell lines, and additional treatment schedules, including those that require S9 activation system(s).
A Three-tiered Approach Linking Pharmacokinetics to Adverse Outcome Pathways for Chemical-Specific Risk Assessment

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The Adverse Outcome Pathway (AOP) framework utilizes a pathways-based approach to provide a biological context for interpreting high-throughput (HT) toxicity screening results. AOPs begin with the perturbation of a molecular target (i.e., molecular initiating event) related to an assay technological target, followed by a series of key events across multiple levels of biological organization, and end with an adverse outcome relevant to regulatory purposes. An AOP itself is not chemical-specific. However, application of the AOP framework and HT predictions in ecological and human health risk assessment requires consideration of chemical-specific properties that mediate external exposure and target tissue doses. To address this requirement, a three-tiered approach was developed to link biology-based AOPs to biochemical-based pharmacokinetic properties (absorption, distribution, metabolism, excretion; ADME), and activity-based exposure pathways associated with chemical behavior and human activity. This tiered approach involves: (1) Qualitative refinement of HT results through identification of false positives (in vitro active chemicals unable to access the molecular target in vivo) and false negatives (in vitro inactive chemicals that are progenitors of active metabolites in vivo); (2) Quantitative scoring and ranking of chemicals based on estimated rates of ADME behaviors acting as surrogates for target tissue doses; and (3) Quantitative modeling to prioritize chemicals by incorporation of information describing hazard potencies, environmental exposures, and chemical-specific ADME characteristics. Application of these tiers is demonstrated in three case studies focused on AOPs related to acetylcholinesterase inhibition and thyroid peroxidase inhibition. This approach provides investigators with multiple tools for applying the AOP framework in chemical risk assessment based on available exposure and ADME information and highlights data gaps and critical needs regarding such information for regulatory decision makers. Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy.
Poster Abstracts

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Read-outs from publicly accessible datasets of validated *in vitro* methods and data generated from several *in silico* software packages were used as input descriptors for the generation of decision tree models for the prediction of skin sensitisation hazard in accordance with EU and GHS classification criteria. Considering the differences in size of the *in vitro* datasets for DPRA, h-CLAT and KeratinoSensTM, a systematic modelling approach was applied in order to generate models from the largest datasets possible including *in vitro* data and to better understand the contributions of the individual methods in the generated models.

In order to develop easily interpretable classification models capable of distinguishing between sensitisers and non-sensitisers, a decision tree approach was adopted. Consistent with the identification of the covalent modification of skin proteins as being the molecular initiating event in the skin sensitisation adverse outcome pathway (AOP), *in silico* and *in vitro* methods measuring reactivity showed the highest power in discriminating sensitisers from non-sensitisers, and were selected as the first decision node in all the trees. Surprisingly, physicochemical properties were usually not included in the decision trees, and *in silico* descriptors were often selected for their discriminatory power over *in vitro* readouts.

A consensus of decision trees showing very high prediction performance (accuracy>0.93 and sensitivity>0.98) is proposed as an Integrated Testing Strategy (ITS) for skin sensitisation classification. Further work is ongoing to explore the feasibility of extending this approach from a simple classification model to one that also predicts the probability of being a skin sensitiser. This presentation will explain the data integration and modelling approach, including the chemical and biological applicability of the resulting ITS for the regulatory assessment of chemicals.
Modeling Ozone Exposure Response Heterogeneity
In Vitro: The Intersection of Inter-individual Variation and the Epigenome

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Traditional toxicity assessments have focused on the effects of exposure to individual pollutants; however, real-world exposures often involve complex toxicant mixtures and multiple exposures. Studying these scenarios by controlled human or animal exposure is limited by cost, time, and practicality. Despite these issues, further study of complex exposures is needed because they are realistic and can produce different outcomes than might be predicted from single, acute exposures. Ozone is a ubiquitous air pollutant that may elicit this type of response. An acute exposure to ozone induces a marked pro-inflammatory response; however, upon multiple exposures this response is attenuated. This ‘adaptive’ response has been consistently observed in vivo, but the underlying mechanism remains unclear. To explore acute and adaptive pro-inflammatory responses to ozone exposure, we developed an in vitro model using fully-differentiated primary bronchial epithelial cells grown at an air-liquid interface. Unlike traditional cell lines, these cultures are stable for weeks to months, lack confounding genetic aberrations, and can be collected from multiple donors. We utilized this model to both assess inter-individual response variability and identify the epigenetic mechanisms underlying the acute and adaptive responses using Nanostring expression profiling and chromatin immunoprecipitation (ChIP), respectively. Repeated ozone exposure produced gene expression profiles distinct from single exposures, which were primarily characterized by the down-regulation of a core set of pro-inflammatory genes (IL-8, IL-6, COX-2, and HMOX-1), mimicking in vivo adaptation. We sought to determine whether baseline differences in the epigenome, the master regulator of gene expression, could explain response variability between donors. We examined epigenetic factors, specifically histone modifications, at the promoters of pro-inflammatory genes and found that the extent of activating marks (such as histone acetylation) was related to gene expression profiles, and that during acute and adaptive exposures the abundance of these marks changed. The ability to relate novel epigenetic endpoints to inter-individual differences in responsiveness demonstrates the promise of in vitro models for studying response mechanisms while recapitulating genetic variation. Ultimately, models such as this one will improve our ability to predict susceptible populations and identify strategies for mitigating the health effects associated with single and complex exposures.
**In Vitro to In Vivo Extrapolation: Optimizing Parameters for Improved Predictions**

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*In vitro* assays provide an efficient way to identify estrogen-active chemicals. However, nominal *in vitro* assay concentrations of a chemical may not accurately reflect the blood or tissue levels that cause *in vivo* effects, mostly due to differences in bioavailability and clearance between the two systems. We evaluated the impact of critical pharmacokinetic (PK) parameters and dosimetry in two models for *in vitro* to *in vivo* extrapolation of activity in the estrogen receptor (ER) pathway. A simple one-compartment PK and a multi-compartment physiologically based pharmacokinetic (PBPK) model were used to correlate *in vitro* effective concentrations to *in vivo* blood concentrations for two reference estrogens and eight environmental chemicals with ER agonist activity. Data from 16 *in vitro* ToxCast assays that map to various key events along the ER pathway (including binding, transcription, and cell proliferation) were used in these models to estimate daily equivalent administered doses (EADs) that would result in a steady-state blood concentration (Css) or maximum blood concentration (Cmax) equivalent to lowest effective concentrations (LECs) in these assays. The EAD estimates from both models were then compared to the lowest effect levels (LELs) for each chemical in *in vivo* uterotrophic assays. We examined the impact of two key PK parameters in relating the administered dose to blood concentration: fraction of unbound chemical (Fub) and hepatic clearance. We systematically varied the values of these parameters across a range of experimentally observed values and investigated the changes in EAD estimates. In addition, to better estimate the free fraction of a chemical that is available for uptake into tissue/cells and results in a biological effect, we applied a Fub adjustment method to estimate EADs that could lead to blood concentrations of free chemicals equivalent to *in vitro* LECs. The models performed well in predicting *in vivo* lowest LELs from *in vitro* LECs for the majority of chemicals tested, particularly after Fub adjustment. This study demonstrates the ability to quantitatively predict *in vivo* effects for proper interpretation of *in vitro* data. *This project was funded in whole or in part with Federal funds from the NIEHS, NIH under Contract No.HHSN27320140003C.*
**Survey of Ecotoxicologically-relevant Reproductive Endpoint Coverage Within ECOTOX Database Across ToxCast ER Agonists**

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The U.S. EPA’s Endocrine Disruptor Screening Program (EDSP) has been charged with screening thousands of chemicals for their potential to affect the endocrine systems of humans and wildlife. *In vitro* high throughput screening (HTS) assays have been proposed as a way to prioritize chemicals for EDSP Tier 1 screening. There are 18 HTS assays within ToxCast that measure chemical bioactivity at different sites along the estrogen receptor pathway. Recent work has correlated these *in vitro* results to *in vivo* estrogenic endpoints, including the rodent uterotrophic assay. It is unclear how the *in vitro* HTS assays, generated in mammalian cell-lines and mammalian receptors, correlates with *in vivo* effects in environmentally relevant species, like fish. Here, ecotoxicological reproduction data from EPA’s ECOTOX database was surveyed across the 91 chemicals identified as ER agonists by the ToxCast ER Model. In total, the ECOTOX database contains over 780,000 entries representing ~11,000 chemicals, ~11,700 species, and ~3300 endpoints. Of the 91 chemicals, 67 had ecotoxicologically-relevant reproductive endpoints within ECOTOX. Endpoints plausibly linked to estrogen-disruption were identified including: vitellogenin synthesis, abnormal sexual development, imposex/intersex conditions, effects in progeny counts, and alterations in population sex ratios. The current survey of ecological study and endpoint coverage represents a valuable resource for the modeling community. *This abstract does not necessarily represent U.S. EPA policy.*
Skin sensitization is an adverse outcome that has been well studied over many decades. It was summarized using the adverse outcome pathway (AOP) framework as part of the OECD work programme (OECD, 2012). Currently there is a strong focus on how AOPs can be applied for different regulatory purposes including the development and application of Integrated Approaches to Testing and Assessment (IATA). One example is an Integrated Testing Strategy developed by Jaworska et al (2013) known as ITS-2 which was derived using a Bayesian network and which relied upon information generated from different in vitro and in chemico assays that characterized the key events within the AOP. Here we evaluated the performance of the ITS-2 model on a separate set of 50 compounds containing sensitizers and non-sensitizers. We explored replacing TIMES-SS, a commercial expert system with the freely available OECD Toolbox Protein binding alerts and re-deriving the model resulted in comparable predictive performance. We also examined whether penetration, expressed as a percentage of the total amount, is a relevant predictor of skin sensitization potential and potency. General dogma supposes size and hydrophobicity as modelled by MW and LogKow are important parameters for evaluating penetration, with a MW>500 often being cited as a threshold for skin sensitization. Roberts et al (2013) examined the training set within TIMES-SS and the extent to which substances with a MW > 500 were sensitizing. Their dataset was limited with 13 compounds above a MW of 500 and of those only 5 were sensitizers. Here we present preliminary findings using the ECHA REACH dissemination dataset which identified 176 compounds with a MW greater than 500 and of those 31 were sensitizers. The findings confirm those of Roberts et al. (2013) and provide greater confidence that penetration is not a relevant predictor for skin sensitization.

The views expressed are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.
**Employing the Metabolically Competent HepaRG Human Cell Line in the In Vitro γH2AX Assay by High Content Screening**

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The lack of metabolic competency of *in vitro* cell systems could lead to the incorrect toxicological evaluation of compounds. For example, pro-toxicant compounds need bio-activation to exert their toxicity. Using metabolically competent cell systems in *in vitro* assays could improve the sensitivity and specificity of the methods. The *in vitro* γH2AX assay was developed on a High Content Screening platform to automatically detect and quantify the phosphorylation of histone γH2AX in response to DNA double-strand breaks (DSBs), an early marker of DNA damage.

Here, two carcinogenic pro-toxicants, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and benzo[a]pyrene (B[a]P), were selected for proof of concept. Levels of DSBs were detected by immunostaining using a fluorophore-coupled γH2AX-specific antibody followed by image acquisition and software analysis on the Cellomics Vti Arrayscan.

After 3 hour treatment, both pro-toxicants produced a positive response in HepaRG cells (γH2AX ≥ 1.5-fold increase over vehicle control and cell viability 25%). NNK produced a positive response at the maximum dose tested (1mM) without any reduction in cell viability. B[a]P produced a positive response from concentrations above 16μM with a reduction in cell viability up to 42%.

Our results indicate that HepaRG cells could be a suitable cell system for the *in vitro* γH2AX assay by HCS, supporting the detection of pro-toxicants causing DSBs. This approach could substitute the use of standard animal-derived S9-mix in *in vitro* assays for hazard identification purposes. More pro-toxicants with different mechanisms of action need to be assessed in this optimised method to evaluate its predictive potential.
Zebrafish Models For Human Acute Organophosphorus Poisoning

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Organophosphorus compounds (OP) are currently the most commonly used pesticides in the world and its use might result in acute poisoning, with around 3 million cases and 300,000 deaths annually. The molecular initiating event leading to organophosphorus compounds poisoning is the inhibition of acetylcholinesterase (AChE), resulting in the accumulation of the neurotransmitter acetylcholine (ACH) in the cholinergic synapses. However, the pathways conducting to some of the adverse outcomes are not well-understood. Zebrafish is being increasingly used as a model species for both ecological and human health risk assessment. Although the modes of action (MoA) leading to toxicity for these types of pesticides have already been analyzed in zebrafish, these analyses have been performed during embryonic development and therefore, are more related with the developmental toxicity than with the OP poisoning. In this study we use a prototypic OP compound, chlorpyrifos-oxon (CPO), to develop a zebrafish model of OP poisoning using a set of molecular markers. The acute effects of mild, moderate, and high CPO concentrations on zebrafish larvae have been studied at different levels of organization including molecular (transcriptomic analysis by RNAseq, biochemical responses), cellular, tissue and organismal (gross morphology, behavioral effects [visual motor response (VMR) and touch-evoked escape response]). Three phenotypes were identified by gross-morphology and behavioral analysis. In conclusion, the adverse outcome pathways of the different phenotypes have been dissected empirically and the results obtained show that exposed zebrafish mimic many aspects of the human OPP, therefore confirming the use of zebrafish larvae as a suitable model to identify new antidotes against cholinergic syndrome.
**A Framework for Rapid Hazard Assessment of Chemicals Using Quantitative Adverse Outcome Pathways**

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The increasing number of new and existing chemicals being developed and released into the environment requires new approaches for early assessment of the potential hazards and adverse effects that these materials pose to both humans and the environment. Hence there is a great need to rapidly assess these chemicals. Here, we present a chemical hazard testing framework that utilizes a combination of chemical modeling, concentration-responsive effects assessment, *in vitro* to *in vivo* extrapolation, and a quantitative Adverse Outcome Pathway (AOP) approach to integrate data and derive a useful measure of chemical hazard. Quantitative Structure Activity Relationships (QSAR) are used initially to predict acute aquatic toxicity levels. The toxicity of a chemical will then be examined through exposure of zebrafish embryos to 5 different concentrations of the chemical. Effects of the chemical are assessed morphologically and transcriptionally to identify potential toxicity pathways and target tissues. Concentration-dependent changes in gene expression will then be incorporated into the AOPXplorer, a tool that allows us to integrate, explore, and visualize the data in ways that will help us understand what the molecular responses mean within a larger biological context, and to facilitate the automated screening of hazards and perform dose-response assessments in a more rapid fashion. We expect that this framework will provide more accurate hazard level screening tools and enable the reduction and focusing of *in vivo* safety tests required for new chemical development resulting in significant savings in money and time.
**In Silico Modeling Workflows in Support of Exploratory Computational Toxicology**

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Integrating *in silico*, *in vitro* and *in vivo* data into a rational toxicoinformatic workflow to inform and support risk-assessment is a major scientific undertaking that can only be facilitated by quality data, and robust computational tools. The ToxCast/Tox21 dataset in the iCSS (Interactive Chemical Safety for Sustainability) dashboard is an ideal dataset for *in silico* model development to support the rational molecular-based screening and prioritization of bioassay properties. Combined *in silico* and *in vitro* techniques have previously been used to explore nuclear receptor-based binding properties (e.g., ER, AR and TR) for existing endocrine panels. The present work demonstrates the development of a variety of 1D, 2D and 3D *in silico* “nuclear receptor superfamily” models generated using the Molecular Operating Environment (MOE 2014.09: Chemical Computing Group, Montreal Canada) in conjunction with end-point specific iCSS data, and structural data from MOE Protein Families and the RCSB.

The scope of the data development ranges from ligand/protein structure preparation for *in silico* data generation (such as molecular docking studies) to QSAR-derived descriptor-based filters and classifiers (for apical endpoint estimates and targeted protein-ligand structure-based classifiers) which include both qualitative and quantitative *in silico* toxicogenomic datasets and workflows.

These models and underlying molecular toxicoinformatics-driven workflows can be used independently, or in tandem with other techniques that have been developed, to provide rapid assay-chemical and target-toxicant relationship networks for interpreting adverse outcome pathways (AOPs) and to gain toxicological insight with a molecular and atomic level of resolution.
Advances in In Vitro Testing: The Route to Replace Animal Testing

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The pharmaceutical industry is coming under increasing pressure to overhaul the drug discovery and development process. The challenge is to fix the high rate of attrition, whereby too many promising drug candidates fail at late stages in the process. Although assays developed using static cell culture techniques can provide valuable insights, the limitations of these systems in only partially replicating normal biological processes impacts on the utility and reliability of the resultant data.

Advances in the area of in vitro testing offer a promising solutions to tackle this attrition problem. In vitro models have become more sophisticated and are now at a stage where they can provide an effective alternative to some in vivo experiments. Creating an in vitro environment which encourages cells to behave in the way they do in their natural situation allows for the development of more predictive models as well as providing the framework to capture more of the complexities of normal biological function and replicate inter-individual variability. Cells experience nutrient gradients, pressure and flow, and they communicate with each other. Cells cultured in static media in well plates, whether primary cells or cell lines, cannot replicate this highly organised structure. Quasi-Vivo® perfusion systems – which provide nutrient flow and allow for co-culture and the development of 3D structures – are delivering improved viability and function. Cells cultured in this environment have been shown to retain phenotype and exhibit long-term metabolic competency. This offers more meaningful data from in vitro assays. Preliminary results are presented that illustrate the potential for advanced in vitro cell culture, including live cell imaging in the QV900. The Quasi-Vivo® perfusion systems provide a stepping stone towards the more speculative organ-on-a-chip or human-on-a-chip technology.

A key part of the strategy to replace animal testing is to find some common types of experiment that can be done better in vitro than in vivo, and that will result in both scientific and ethical gains. This paper will include ‘case studies’ that illustrate how culture models can be used to answer a range of important biological questions of direct relevance to human development, disease and healing.
**NTP Tox21 Toolbox to Prioritize Chemicals for Extensive Toxicological Testing**

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The U.S. Tox21 program is currently screening a 10K compound library using a battery of assays as surrogates of toxicity pathways, via quantitative high through put screening (qHTS). To date, more than 70 assays have yielded >12 million concentration-response curves. In order to visualize and analyze these data, several web applications have been developed by the NTP. These tools are of two types: compound qHTS activity characterization and compound biological function inference. The Tox21 Activity Profiler and Tox21 Actives allow the user to explore the compound qHTS activity results coming from two different statistical data analysis pipelines (“Weighted Area-Under-Curve” and “Curve Class”). The Tox21 Curve Browser visualizes concentration-response curves for all Tox21 assays, and provides concentration-response data for download. The Tox21 Correlation Browser and Tox21 Enricher can be used in combination to infer biological/toxicological mechanism, based on chemicals’ patterns of responses in multiple assays. Specifically, the Tox21 Correlation Browser identifies subsets of chemicals that exhibit correlated assay response patterns to individual Tox21 chemicals entered as query. Resulting lists of correlated chemicals can then be evaluated for chemical annotation enrichment using the Tox21 Enricher. The enrichment results provide a hypothesis as to the biological or toxicological properties of the group of correlated chemicals together with the query chemical. Current chemical annotation categories available in the Tox21 Enricher include KEGG pathway, and Gene Ontology (GO) from Comparative Toxicogenomics Database (CTD); predicted in vivo toxicity, and chemical substructures provided by Leadscope, Inc.; a number of clinical and toxicological classifications from DrugMatrix, and PubChem MeSH terms. These tools (italic) are available at http://ntp.niehs.nih.gov/tbox/. Ongoing development is focused on applications to prioritize compounds without query compound specification, incorporation of chemical annotations from additional databases, and integration of results from different data analysis pipelines into the NTP Chemical Effects in Biological Systems (CEBS) database.
A Multi-scale Network Perspective on the Aryl Hydrocarbon Receptor Toxicity Pathway

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I will present the results of recent analysis of the transcriptional regulatory network underlying the arylhydrocarbon receptor (AhR) toxicity pathway in mouse liver. We have used a combination of gene expression and genome-wide transcription factor binding data to derive the regulatory network. Superposing gene expression time course data on the network revealed the regulatory logic and sequential activation among the nodes in the network. Analysis of the AhR network with Kohonen self-organizing maps and subspace clustering algorithms showed significant overlap between gene co-regulation and gene co-expression, while Lasso regression identified the transcription factors most predictive of gene expression patterns. Gene ontology analysis revealed differential activation of several groups of biological processes, including metabolism, immune response and kinase activation. This network mapping and modeling procedure can be used to support a quantitative framework for safety assessment of environmental chemicals acting through core toxicity pathways.
Steroidogenesis, including both steroid hormone biosynthesis and metabolism, is critical for proper endocrine function. Disruption of steroidogenesis by environmental chemicals results in altered hormone levels that may cause adverse reproductive and developmental effects. This study presents a high-throughput adaptation of the OECD validated H295R human adrenocortical carcinoma steroidogenesis assay. A 96-well format and hormone quantification by HPLC-MS/MS allowed for the evaluation of a diverse library of chemicals for effects on 13 major hormones in the steriodogenic pathway. Steroidogenesis was induced by pre-stimulation with 10 μM forskolin for 48 hr followed by chemical exposure for 48 hr. Media were removed and quantification of progestagens (pregnenolone, progesterone, and their hydroxylated metabolites), glucocorticoids (corticosterone, cortisol, and their deoxy-precursors), androgens (dehydroepiandrosterone, androstenedione, and testosterone), and estrogens (estrone and estradiol) was conducted. Initially, nearly 2000 ToxCast chemicals were tested at a single non-cytotoxic concentration, of which 936 chemicals altered levels of at least one measured hormone. Based on the single concentration analysis, 395 chemicals altering the levels of ≥4 hormones were selected for six-point concentration-response evaluation (0.003 – 100 μM). Compared to results from OECD guideline criteria for this assay, which requires only changes in testosterone and/or estradiol, our criteria of ≥4 altered hormones identified chemicals with 91% sensitivity. Furthermore, the profiles generated by quantifying 13 hormones in concentration--response not only characterized chemical-elicited disruption in steroidogenesis, but also identified distinct putative modes of action. For example, distinct patterns of decreased glucocorticoid levels with concurrent increases in progestagen levels distinguished putative HSD3B inhibition. Additionally, increased progestagen levels along with decreased androgen and estrogen levels was observed for putative CYP17A disruptors. These data suggest that a high-throughput adapted evaluation of steroidogenesis using the OECD validated H295R platform can provide additional insight into chemical-mediated effects on steroidogenesis. This abstract does not necessarily reflect US EPA policy.
Identifying Reference Chemicals for Androgen Receptor Activity

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Mandated testing of thousands of chemicals to identify those that may act as androgen receptor agonists or antagonists will cost millions of dollars and take decades to complete using current validated methods. Alternative methods using high-throughput screening and computational toxicology technologies, such as those developed in the ToxCast and Tox21 screening programs, can provide rapid and cost-effective identification of potential androgen-active chemicals. Development and evaluation of alternative test methods and testing strategies will require high-quality reference data from in vivo and in vitro assays for anti/androgenic activity. To focus the search for quality in vitro data, a list of 121 putative androgen-active or inactive chemicals for which ToxCast data were available was compiled from previous ICCVAM, EC-VAM, and OECD validations of in vitro androgen receptor binding and transactivation assays. We conducted semi-automated literature searches for in vitro androgen activity data on these chemicals using PubMatrix and Scopus. High-quality in vitro binding and transactivation data were extracted from identified references and compiled into a single database, which will be publicly available on the NTP website (http://ntp.niehs.nih.gov/go/40658). Detailed assay information and results were recorded in the database using a standardized ontology. These data were analyzed for reproducibility, consistency, and specificity of results across assay systems and receptor types. Antagonist data were only considered in the analysis if cytotoxicity was evaluated concurrently. Based on quantitative data such as relative binding affinity and transactivation activity concentrations, chemicals with reproducible results were assigned potency ranges. Reference chemical lists and supporting documentation resulting from this effort will be made available to the public and submitted to OECD via the Validation Management Group-Non Animal to facilitate the international harmonization of test method evaluations. This work does not reflect EPA policy. This project was funded in whole or in part with Federal funds from the NIEHS, NIH under Contract No.HHSN273201500010C.
Update for Japanese Project “ARCH-Tox” for the Future Chemicals Management Policy: Research and Development of In Vitro and In Vivo Assays for Internationally Leading Hazard Assessment and Test Methods

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In 2011, Japan’s Ministry of Economy, Trade and Industry (METI) launched a new 5 years research project, entitled as “ARCH-Tox”, with the goal of promoting the 3Rs in 28-day repeated dose oral toxicity studies, which are used to screen for compliance with Japan’s Chemical Substances Control Law. This project includes the following two sub-projects:

1. Tox-Omics Project: Development of methods to detect multiple-toxic effects using gene expression analysis. This project is developing the methodology obtaining the biological information that lead to toxicity from gene expression profiles in animals tested in 28-day repeated dose studies (28-d RDS). The project has developed the prediction systems for carcinogenicity and detection systems for toxicities in liver and kidney by using the gene expression profile obtained in 28-d RDS. The detection systems for neurotoxicity, and the refinement and mechanistic analysis of the developed systems are currently underway.

2. Tox-In vitro Project: Development of in vitro assays to detect toxicities, including target organ toxicity and metabolic function. This sub-project will attempt to establish in vitro test methods simulated in vivo toxic effects for the speedy and efficient assessment of hepatotoxicity, nephrotoxicity, and other endpoints in repeated dose studies. This project produced transgenic cells that detect expressions of toxicity marker genes by convenient multi-luminescence reporters using mammalian artificial chromosome vectors. Now this project is developing the in vitro assay system to detect hepatic toxicity, renal toxicity and neurotoxicity using these reporter cells.

We believe that the successful completion of these projects will help further worldwide application of the 3Rs to safety evaluation of chemicals in systemic toxicity testing.
Developing a Physiologically-Based Pharmacokinetic Model Knowledgebase in Support of Provisional Model Construction

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Building new physiologically based pharmacokinetic (PBPK) models requires a lot of data, such as the chemical-specific parameters and in vivo pharmacokinetic data. Previously-developed, well-parameterized, and thoroughly-vetted models can be a great resource for supporting the construction of models pertaining to new chemicals. Thus, a PBPK knowledgebase containing existing PBPK-related articles was compiled. From the analysis of 2,039 PBPK-related articles, 307 unique chemicals were identified in 795 publications. Keywords related to species, gender, developmental stages, and organs were analyzed from these 795 articles. These articles together with the chemical names, species and other indexes were included in the PBPK knowledgebase. In addition, a correlation matrix of the 307 chemicals in the PBPK knowledgebase was calculated based on their pharmacokinetic-relevant molecular descriptors. Two case studies were conducted to demonstrate the utility of the knowledgebase. In these case studies, all chemicals in the PBPK knowledgebase were ranked based on their correlation toward ethylbenzene and gefitinib. Next, chemicals from the upper and lower portion of the ranking list were selected to represent exact matches, close analogues, or non-analogues of the target chemicals. Parameters, equations, or experimental data relevant to existing models for these chemicals were used for new model construction and predictions. This compiled knowledgebase provides a chemical structure-based approach for identifying PBPK models relevant to other chemical entities. Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy.
Domain-Specific QSAR Model for Identifying Potential Estrogenic Activity

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Humans are potentially exposed to tens of thousands of man-made chemicals in the environment, some of which may mimic natural endocrine hormones and thus have the potential to be endocrine disruptors. Predictive in silico tools allow quick and efficient evaluation of untested chemicals for their ability to disrupt the endocrine system. The Collaborative Estrogen Receptor Activity Prediction Project (CERAPP) brought together international experts to build an ensemble of models to identify chemicals with the potential to interact with the estrogen receptor (ER). QSAR models from the different groups were trained and validated on the ToxCast/Tox21 ER assay data, and then used individually and in combination to screen a large library of ~30,000 chemicals for ER binding and agonist activity. The CERAPP results showed a high prevalence of phenolic compounds in the set of predicted positives, consistent with prior knowledge on the influence of this structural moiety on chemical interaction with the ER. However, because CERAPP global models did not accurately predict specific activity and relative potency of various phenols, we constructed local QSAR models focused on this chemical category. Phenolic compounds were identified in the curated CERAPP dataset by the presence of a benzene ring with a hydroxyl substituent (aryl ethers were not considered.) Machine learning approaches, such as random forest and partial least squares discriminant analysis, were applied to build local QSAR models for ER binding and agonist activity of phenolic compounds. These models were trained and tested on data from the ToxCast/Tox21 assays and well-curated literature sources with independently reproducible results. The local models consistently yielded higher predictivity (balanced accuracies ~0.9) and better balance between sensitivity and specificity than the global models as evaluated by their performance on the external test sets. These models can be used as support tools for evaluating the endocrine disrupting potential of environmental chemicals. 

This work does not reflect EPA policy. This project was funded in whole or in part with Federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.
Exposure to Nicotine Does Not Mimic the Effects of Total Particulate Matter on Early Development of Zebrafish (Danio rerio)

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Cigarette smoke has been associated with a number of pathologies; however, the mechanisms leading to developmental effects are yet to be fully understood. This study examined the effects of Total Particulate Matter (TPM) from 3R4F reference cigarettes on the early development of zebrafish (Danio rerio). Zebrafish embryos were exposed to two concentrations of TPM (0.4 and 1.4 μg/mL equi-nicotine units). Nicotine-alone exposures at equivalent doses were also performed. The exposures (single and acute) began at 2 h post fertilization (hpf) and lasted until 96 hpf. Several physiological parameters were assessed during or after the exposure. We show that TPM, increased mortality, delayed hatching, and increased the incidence of deformities in zebrafish. TPM exposure also increased the incidence of hemorrhage and disrupted the proper angiogenesis of the major vessels in the brain. Moreover, TPM exposure reduced the larval body length, decreased the heart rate, and reduced the metabolic rate. Biomarkers of xenobiotic metabolism and oxidative stress were also affected. TPM-exposed zebrafish also differed behaviorally: at 24 hours post fertilization (hpf) the embryos had a higher frequency of spontaneous contractions and at 144 hpf the larvae displayed swimming hyperactivity. This study demonstrates that TPM disrupts several aspects of early development in zebrafish. The effects reported for TPM were not attributable to nicotine, since embryos treated with nicotine alone did not differ significantly from the control group. Collectively, our work illustrates the utility of zebrafish as an alternative model to evaluate the toxic effects of cigarette smoke constituents.
Cytokine Induction in the 3D EpiDerm™ Skin Model used as an In Vitro Preclinical Screening Tool for Formulations with Anti-inflammatory Action

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Abnormal cytokine profiles represent the hallmark of inflammatory skin conditions such as psoriasis, acne, atopic dermatitis, irritant and allergic contact dermatitis. Damage to skin keratinocytes induces the release of primary cytokine interleukin (IL)-1α which further stimulates the release of secondary cytokines (e.g., IL-8) involved in the mediation of inflammatory reactions. Animal models have been historically used to assess the potency of formulations designed to intervene in the inflammatory cascade. In recent years, in vitro testing methods based on three-dimensional (3D) reconstructed skin equivalents became a reliable, rapid tool to screen actives and formulations for efficacy claims, including potential anti-inflammatory action. Here we present data generated in a novel in vitro assay based on the EpiDerm™ Human Cell Construct (MatTek Corporation). The EpiDerm™ tissues were exposed topically for 6 hours to materials intended to counteract the inflammation induced by phorbol-12-myristate 13-acetate (PMA) added to the culture media. Two different ingredients with known anti-inflammatory activity formulated as creams were evaluated (OTC-class 7 low potency, and Rx-class 2 high potency, formulated for augmented penetration). The low potency active was also tested as a spray along with an alcohol-based hand sanitizer. To avoid over-prediction of the irritation, the alcohol-based formulations were applied to the tissues at a reduced dosing volume of 30 μL, while the creams were applied as 100 μL doses. The cytokines analyzed were IL-1α and IL-8 (released in the culture media and in the lysed tissues). Our data showed that IL-1α analyzed in the lysed tissues and IL-8 analyzed in the culture media were reliable indicators of anti-inflammatory actions for the materials tested. Both cytokine indicators showed that the Rx cream formulated for augmented penetration was the most effective of the creams in reducing the cytokines’ levels, thus supporting the class 2 high potency. Furthermore, the class 7 active formulated as a spray had a stronger anti-inflammatory action compared to its cream counterpart despite the reduced dosing volume. Our data support the potential use of the Rx class 2 cream and the OTC class 7 spray as reference materials for screening formulations investigated for anti-inflammatory action.
Use of High-Content and High-Throughput Assays and Predictive Network Analysis in Integrated Testing and Assessment by using HEALS System: Two Case Studies

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Phenotypic analysis in cell-based assay is a powerful approach used in biological research and drug discovery to identify substances. However, the application of this approach should be not yet improved in some toxicology fields. We here introduce two case studies that high-content and high-throughput assays and predictive network analysis were used to identify a new characteristic chemicals in integrated testing and assessment by using HEALS system (http://project.nies.go.jp/heals/), which is a tool for predicting chemical effects. The first case is a new method using potentially sensitive stem cells for assessment of epigenetic effects. We evaluated effects of chemicals on global DNA methylation with mouse embryonic stem cells (ESCs) harboring fluorescence fused Methyl CpG–binding protein 1 (GFP-MBD1) in a high-content and high-throughput assays. GFP-MBD1 has been defined as most selective domain to bind methylation, and applied as a biological sensor for DNA methylation detection. Sensitivity and structure of GFP-MBD1 in nuclei of mouse ESCs were characterized for high-throughput screening. The parameters in mouse ESCs with were validated according to comparison between vehicle control and demethylation agents, histone deacetylase inhibitors and estrogens for direct determination of genomic DNA methylation level. These would enhance the range of safety assessment tools for evaluating environmental chemicals that perturb epigenetics. The second case showed a combination approach of phenotypic assay and chemical analysis to detect low dose mixtures in diet, water and indoor air contaminants. It is need to establish a rapid and simple assessment method because this problem is recognized as an emerging issue under the chemical regulation. We investigated relationships between mixtures and toxicities using a combination of toxicity-based phenotypic analysis and QTOFMS analysis. 17ß-estradiol (E2) or environmental contaminants-induced phenotypic variation of MCF-7 was analyzed by high-content assay. Same samples simultaneously applied to QTOFMS analysis to detect unknown chemical information. Those phenotypic parameters and QTOFMS data were statistically analyzed with Bayesian approach in HEALS. The present results demonstrated good examples in toxicity-based phenotypic analysis.
A Practical Guidance for Cramer Class Determination

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Expanded use of the Threshold of Toxicological Concern (TTC) methodology has brought into discussion the intent of the original questions used in the Cramer scheme or Cramer decision tree. We have analysed a large dataset of fragrance materials and identified several issues with the original Cramer questions. Some of these relate to definitions and wording of questions; some relate to the in silico interpretation of the questions. We have endeavoured to address all of these inconsistencies and misinterpretations without changing the basic structure and principles of the original decision tree. Based on the analysis of a large data set of more than 2500 fragrance ingredients, we have found that most of the 33 questions in the original Cramer scheme are straightforward. However, to be answered correctly, they do require a working knowledge of organic chemistry. Through repeated examination of each of the 33 questions, we found that there are questions where the logic underlying the development of the rule is less than transparent. These questions (e.g., Q9, Q16, Q17, Q22, Q25, Q26 Q30) are well served by minor wording changes and/or further explanation designed to capture what we perceive to be the intent of the original decision tree. The findings reported here could be used as guidance for conducting Cramer classification and provide advice for the improvement of the in silico tools.
Practical Considerations for Routine Screening of Skin Sensitizers using the KeratinoSensTM Assay

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The recent publication of the OECD test guideline for In Vitro Skin Sensitization using the ARE-Nrf2 Luciferase Test Method (Test Guideline 442D) has increased demand for the KeratinoSensTM assay. In the KeratinoSensTM cell-based reporter gene assay, the induction of a luciferase gene under the control of the antioxidant response element (ARE) derived from the human AKR1C2 gene is quantified and cell viability is measured. During routine testing of a wide range of chemicals, it has been observed that certain chemicals prove challenging to assess due to insolubility or interference with assay reagents using standard assay procedures. In this study, we sought to determine if modifications to standard assay procedures could improve predictions for these chemicals. Current guidance only recommends two solvents, water and dimethyl sulfoxide (DMSO). We are investigating additional solvents, including ethanol and acetone, which may improve bioavailability for certain chemicals. In the assay, cytotoxicity of the test substance is assessed using MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). Residual chemical which can directly reduce MTT or interfere with the colorimetric reading may result in an inaccurate assessment of cell viability. We have explored the addition of a rinse step prior to the addition of MTT which has reduced interference and improved measurements. Although the majority of chemicals are not affected by these limitations, exploratory research into these areas may lead to process improvements allowing for application to a wider variety of chemicals.
Identification of Absorption, Distribution, Metabolism, and Excretion (ADME) Genes Relevant to Steatosis using a Systems Biology Approach

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Ensuring chemical safety and sustainability form a main priority of the U.S. Environmental Protection Agency. This entails efforts on multiple fronts to characterize the potential hazard posed by chemicals currently in use and those to be commercialized in the future. The use of an adverse outcome pathway (AOP) framework forms the basis of this strategy, along with exposure characterization. AOPs themselves are meant to be chemical agnostic, but specific chemical exposures and their effects can be informative for discovery and description of AOPs. In order to more comprehensively describe AOPs, we are collaborating on the construction of an absorption, distribution, metabolism, and excretion (ADME) module to the AOP knowledgebase repository. ADME parameters represent important links between exposure and AOP activation in the target tissue. We are specifically interested in identifying relevant genes related to ADME. Our overarching goal is create as comprehensive a list as possible, but to begin we are using Non-Alcoholic Fatty Liver Disease (NAFLD) and hepatic steatosis as a case study. To identify genes related to these conditions, we have utilized the publicly available toxicogenomics database, DrugMatrixTM. This database contains rodent chemical exposure data, along with differential gene expression data and corresponding associated pathology changes. We examined chemical exposures resulting in pathologically confirmed cases of steatosis, and from these exposures, utilized differential and co-expression analyses to identify gene changes resulting from the chemical exposure leading to steatosis. We then utilized pathway enrichment analysis to identify ADME related genes. Our desired product is a comprehensive database encompassing ADME related information, including genes and quantitative models connecting chemical exposure and their potential hazard by interaction with an AOP. The ultimate goal is to increase the speed and decrease the cost of hazard characterization of chemicals of unknown toxicity. This abstract represents the opinion of the authors and does not necessarily reflect U.S. Environmental Protection Agency policy.
**Exposure to Smoke Induces Loss of Bacterial Community Diversity and Inhibits Cellular Stress Response**

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Combustion generates air pollutants like ozone, acrolein, and particulate matter, which can impair normal protective barriers and predispose the airways to bacterial colonization, increasing the risk of infection. Airway infections, such as pneumonia, increase mortality in susceptible populations like burn victims with inhalation injury. To assess the impact of combustion-generated air pollutants on airway bacterial colonization, we characterized the bacterial communities, or microbiota, in the airways of burn patients with inhalation injury. Results from 66 patients indicate that bacterial community diversity decreases with increasing time post injury and with severity of acute respiratory distress syndrome (ARDS). Loss of bacterial community diversity is associated with increased severity of disease and may indicate establishment of infection and poor outcomes in these patients. The airway epithelium is the first line of defense against bacterial infection. To explore the effect of air pollutant exposure on the interactions between the airway epithelium and the microbiome, we developed an *in vitro* model using fully differentiated primary human bronchial epithelial cells (pHBEC) grown at air-liquid interface (ALI). Bacteria can adhere to particles generated during combustion and may be inhaled together. To replicate this *in vitro*, wood smoke particles (WSP) and *K. pneumoniae* were introduced to the cells concurrently. Exposure of pHBEC to WSP led to an increase in expression of hemeoxygense-1 (HO-1), a marker of oxidative stress. By comparison, exposure of HBECs to WSP and *K. pneumoniae* attenuated induction of HO-1 expression. HO-1 down-regulation may imply an interaction between WSP and *K. pneumoniae* that attenuates HBEC response to bacteria and predisposes the airways to infection. These results demonstrate that particulate matter induces markers of oxidative stress response in vitro and alters colonization patterns of bacteria *in vivo*. *In vitro* co-exposure to particulate matter and bacteria indicates that the presence of bacteria can inhibit the cellular stress response, which is likely to alter the ability of the airway epithelium to respond to bacterial exposure. Understanding the interaction between combustion products and bacteria may reveal markers of exposure based on bacterial community composition and therapeutic targets to decrease the risk of pneumonia in exposed populations. *This work does not reflect EPA policy.*
Fast and cost-effective approaches are needed to evaluate the potential of thousands of man-made chemicals to disrupt the endocrine system, including interaction with the androgen receptor (AR) pathway. The Tox21 and ToxCast programs have tested ~1800 chemicals in a broad panel of in vitro high-throughput screening (HTS) assays. Nine assays that map to the AR pathway have been integrated into a computational model to identify substances with potential androgenic/anti-androgenic activity in vivo. The present work uses the HTS dataset, the associated computational model output, and machine learning methods to develop quantitative structure–activity relationship (QSAR) models to predict AR antagonism. Although most ToxCast chemicals (1517) were predicted to be inactive against the pathway, 225 were predicted to have some antagonist activity. QSAR classification models were built to relate the molecular structures of chemicals to predicted AR activities using linear discriminant analysis, classification and regression trees, and support vector machines (SVM) with 51 molecular descriptors from QikProp and 6293 structural fingerprints as potential variables. A random forest (RF) feature selection method was used to extract structural features most relevant to AR activity. A training set of 1161 chemicals was used to derive and optimize the binary classification models. A test set of 581 chemicals was used to validate the performance of each model for overall accuracy, sensitivity, specificity and G-mean. In addition to binary classification, the models predicted potency of the 225 active compounds using multiple linear regression and partial least squares regression. The best performing model was obtained using SVM in combination with a subset of descriptors identified via the RF algorithm. This model was then used to make predictions for a broader chemical universe, predicting that 20.6% (6475/31428) of these chemicals may have AR antagonist activity. This initial result is certainly an overestimate that is confounded by very weak activity or cytotoxicity, and further refinement of the model should improve specificity. This project was funded in whole or in part with Federal funds from the NIEHS, NIH under Contract No.HHSN273201500010C.
Speaker Bios
**J. Craig Rowlands, Ph.D.**

Dr. J. Craig Rowlands is Senior Scientist and Research Leader at The Dow Chemical Company department of Toxicology, Environmental Research and Consulting. He holds a B.S. in Biochemistry and Ph.D. in Molecular Toxicology from Texas A&M University and completed a fellowship in Molecular Endocrinology at the Karolinska Institutet in Stockholm, Sweden. Dr. Rowlands has published over 80 articles and book chapters and edited several reports in toxicology and risk assessment. His current research efforts are in the areas of systems biology and toxicology with a focus on sustainability and the development of non-animal alternative testing strategies towards reducing environmental, health and safety risks of chemicals. Dr. Rowlands has served as President of the Molecular and Systems Biology Specialty Section of the Society of Toxicology, and Chair of the Society of Toxicology Continuing Education Committee. He serves on the Board of Trustees of the International Life Sciences Institute (ILSI), Health and Environmental Sciences Institute (HESI), Co-Chairs the ILSI-HESI project Framework for Intelligent Non-Animal Methods for Safety Assessment and serves on the Society of Toxicology, Current Concepts in Toxicology Committee. As a fellow at the FDA-CFSAN, he oversaw a Food Advisory Committee on Health Claims, and was a co-developer of the Evidence-Based Review System for the Scientific Evaluation of Health Claims. He is on the editorial board of the Journal of Biochemical and Molecular Toxicology. Dr. Rowlands has been awarded the Carl C. Smith award for meritorious research from the Society of Toxicology Mechanisms Specialty Section, and the 2012 Michigan Green Chemistry Governor’s Award. Dr. Rowlands is an adjunct Professor of Toxicology at Michigan State University, Diplomate of the American Board of Toxicology, and a Fellow of the American College of Nutrition.

**Warren Casey, Ph.D.**

Dr. Casey is the Director of National Toxicology Program’s Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), part of the National Institute of Environmental Health Sciences (NIEHS). Prior to assuming his current position, Dr. Casey worked in the pharmaceutical industry for 15 years at GlaxoSmithKline in a variety of areas, including: Microbiology, Toxicogenomics, Investigative Toxicology, Discovery and Molecular Toxicology, and Biomarker Development. Dr. Casey received his undergraduate degree in biochemistry and his PhD in microbiology from North Carolina State University, where he has been named a Distinguished Alumnus and also holds an adjunct professorship in the Department of Microbiology. He has been a Diplomate of the American Board of Toxicology (DABT) since 2007.
**David Dix, Ph.D.**

Since May of 2013, Dr. David Dix has served as Director of the Office of Science Coordination and Policy (OSCP) of the U.S. Environmental Protection Agency (EPA) in Washington DC. OSCP provides coordination, leadership, peer review, and synthesis of science and science policy for EPA’s Office of Chemical Safety and Pollution Prevention, assuring sound scientific decisions and coordinating emerging exposure and hazard assessment topics, such as endocrine disrupting chemicals. Prior to joining OSCP, Dr. Dix was Deputy and then Acting Director of EPA’s National Center for Computational Toxicology in Research Triangle Park, NC, where he led development of high throughput decision support tools for chemical exposure, hazard and risk. Dr. Dix joined EPA’s Office of Research and Development in 1995 as a Research Biologist, leading studies in reproductive, genomic and computational toxicology. He is an Adjunct Professor in the Department of Environmental Sciences and Engineering at the University of North Carolina at Chapel Hill. Dr. Dix earned a B.S. in Biological Sciences from the University of Illinois, a Ph.D. in Physiology from Rush University in Chicago, followed by postdoctoral training at the U.S. National Institute of Environmental Health Sciences. He has published over 120 scientific articles, reviews, reports and book chapters; serves on several journal Editorial Boards; and has represented EPA at a wide range of national and international meetings.

**Joanna M. Matheson, Ph.D.**

Joanna M. Matheson, Ph.D., is a Senior Toxicologist in the Directorate for Health Sciences at the U.S. Consumer Product Safety Commission, an independent federal agency charged with protecting the public from unreasonable risks of serious injury or death from more than 15,000 types of consumer products. Her primary focus is to perform risk assessments relating to toxic exposure from consumer products, particularly to susceptible populations. In addition to her work involving lead in children’s products, she is the project manager for agency’s FHSA definition of “strong sensitizer” and works on Immunotoxicity issues as well as projects involving other substances such as mercury, cadmium and chromium and products such as problem drywall, laminate flooring, and liquid laundry packets. In addition, she serves as the agency liaison to the Federal Liaison Group on Asthma, Federal Interagency Lead Task Force, Federal Committee on Indoor Air Quality, Federal Interagency Committees on the OECD Test Guideline Program and the GHS, ICCVAM, and is co-chair of ICCVAM’s Skin Sensitization Working Group. Her research background focused on the role of immune and inflammatory mediators in occupationally-induced diseases.
Grace Patlewicz is a chemist and toxicologist by training. She started her career as a safety evaluation scientist at Unilever in the UK before focusing her interests in computational toxicology and moving into a role that involved providing modeling and chemistry expertise for a variety of different projects. As the momentum for (Q) SARs to be used for different regulatory purposes was growing in the run up to the EU REACH regulation, she joined the (Q)SAR group at the European Commission’s Joint Research Centre (JRC) in Italy, where she was involved in many activities related to the development of technical guidance for REACH, including investigating the feasibility of using computational approaches in the development of chemical categories, developing and evaluating (Q)SAR models for human health, coordinating the technical development of software tools, such as Toxtree and Toxmatch and contributing to the development of the OECD QSAR Toolbox. In 2008 she returned to Industry taking up a position at DuPont in the US as the focal point and technical lead for all (Q)SAR and read-across queries for product stewardship and regulatory purposes. Externally she also chaired workgroups at Cefic LRI, ECETOC and ACC in the development and application of read-across as well as the application and interpretation of HTS assays and their prediction models in the context of Adverse Outcome Pathways. As part of these activities she represented Industry in the associated OECD workgroups.

In December 2014, she took up a post as a Research Chemist at the US EPA’s National Center for Computational Toxicology where she will be focusing her efforts on enhancing read-across approaches with ToxCast data. Dr. Patlewicz received her PhD in organic chemistry from the University of Santiago de Compostela in Spain, her MSc in Toxicology from the University of Surrey, UK and her BSc (Hons) in Chemistry from the University of Manchester, UK.
Miyoun Yoon, Ph.D.

Miyoun Yoon, Ph.D., serves as Director of The Center for Human Health Assessment at The Hamner Institutes for Health Sciences, and is a Hamner Assistant Investigator. Dr. Yoon’s research has coupled biologically based kinetic modeling with targeted in vitro studies to improve human health risk assessment. In her current research, she works to improve in vitro to in vivo extrapolation modeling in order to estimate safe human exposures for various environmental compounds of human health concern. Dr. Yoon received her Ph.D. in Pharmacy from Seoul National University in Seoul, South Korea. Dr. Yoon’s work has been recognized by the Risk Assessment and Biological Modeling Specialty Sections of the Society of Toxicology as best abstracts and papers and by Toxicological Sciences as honorable mention for board of publications best paper. Dr. Yoon is author/coauthor of more than 35 publications including peer-reviewed articles and book chapters. She has served as guest editor of the special issue of Toxicology on the topic of Quantitative In Vitro to In Vivo Extrapolation: an essential element for in vitro-based risk assessment. Dr. Yoon also has been actively providing training courses for PBPK modeling and its applications. She has been a member of the SOT since 2004 and has served as Councilor of the Biological Modeling Specialty Section. She is also a member of the American Society for Cellular and Computational Toxicology (ASCCT).
Edward Carney Award
Dr. Edward Carney was an active and dedicated member of the American Society for Cellular and Computational Toxicology, and a partner, mentor and friend to many in our fields. His passion and leadership will continue to inspire investigators in *in vitro* and *in silico* toxicology through the Edward Carney Predictive Toxicology Award. This award will be provided to the first author of a winning poster at each ASCCT annual meeting, starting this year. The winner will receive a $500 cash award to assist with travel and/or research expenses.

The 2015 winner will be announced at the member reception the evening of October 1.
Supplemental Information
The American Society for Cellular and Computational Toxicology (ASCCT)

**Mission:**

The ASCCT is a scientific society which provides an organized forum for discussion of cellular and computational toxicology approaches, especially as replacements for animal-based toxicology methods. Through its meetings and activities, the Society facilitates the development, acceptance, and routine use of cellular and computational methods through open dialog between industry, academic, advocacy, and regulatory scientists. The Society strives to include the participation of young scientists to promote their contributions to the field.

**Goals:**

- Facilitate the development, acceptance, and routine use of cellular and computational methods
- Increase the routine application and use of computational and *in vitro* methods for prioritization, classification, and risk assessment purposes
- Foster open dialog between industry, academic, advocacy, and regulatory scientists throughout North America
- Include the participation of young scientists to promote their contributions to the field
- Strengthen cooperation between stakeholders

All Members will receive:

- Quarterly e-newsletter
- Access to a growing library of educational webinars from field leaders
- Discounted subscription rates to the journals ALTEX and Toxicology In Vitro
- Discounted registration for ASCCT events
- News and event updates in the *in vitro* and computational toxicology fields
- The chance to network with regulators, scientists, and policymakers on the cutting edge of non-animal toxicology

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Notes