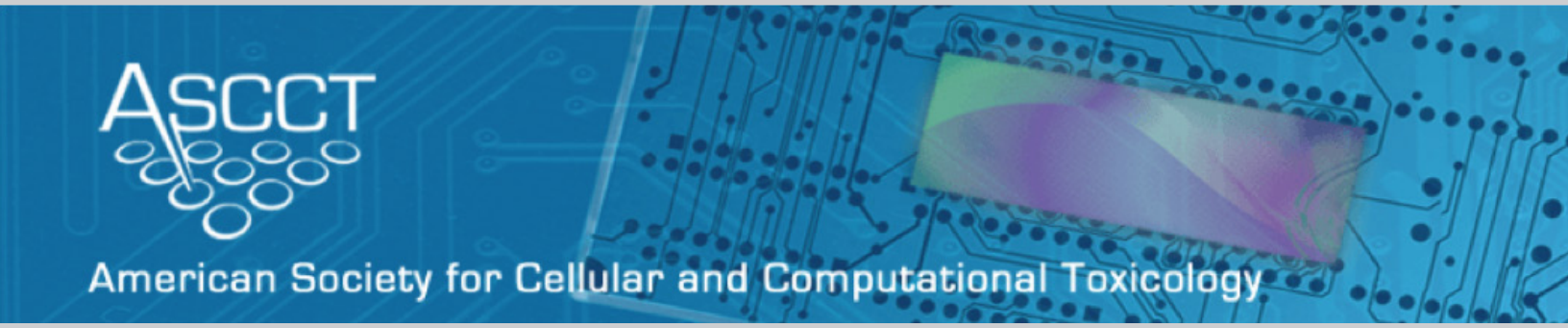

7th Annual Meeting
American Society for Cellular and Computational Toxicology

Predictive Toxicology:
Strategies for Implementing New Approaches

September 11, 2018

Lister Hill Auditorium

Bethesda, Maryland

President's Welcome

Welcome to the 7th Annual Meeting of the American Society for Cellular and Computational Toxicology. We are grateful to NIH for allowing us to bring our meeting to their campus in Bethesda, MD and use their outstanding resources at the Lister Hill Auditorium. Once again, the organizing committee has worked diligently to put together an excellent program around this year's theme Predictive Toxicology: Strategies for Implementing New Approaches. We're excited to have scheduled plenary lectures from leaders in the predictive toxicology field from both government and industry. We also have several platform presentations from ASCCT members that were invited by the program committee based on a review of all submitted abstracts. A panel discussion will close the meeting with a focus on the importance of public-private partnerships to successful implementation of new approach methodologies (NAMs). We've compressed the agenda into one day, but we hope you'll enjoy the full day ahead of exciting new science and how NAMs can impact regulatory decisions.

We'll also recognize two outstanding young scientists with 1) the 4th annual Edward Carney Predictive Toxicology Award, an annual award we started in 2015 to recognize an outstanding poster presentation in memory of our friend and colleague; and 2) the 3rd annual Tox21 Student Award, established in 2016 by our good friend and longtime ASCCT member, Ray Tice. We've also set aside time for a networking event to allow young scientists to interact with many of our more experienced members. And of course, please make sure to take advantage of the poster session during lunch and the reception that will immediately follow the panel discussion later this evening—this year we have 32 posters!

Please take time to thank Kristie Sullivan for her tireless organization and oversight of the outstanding webinars that we've all enjoyed over the past year. We now have 24 webinars in the archive for your reference; what a great a perk to maintaining your ASCCT membership! And we've already got two more scheduled later this fall: An update on progress and near-time applications of AOPs, and the application of stem cell-based models for predicting human tissue injury. And join me in welcoming founding ASCCT member, Erin Hill, to her rightful post as incoming President of ASCCT. I am certain that the society will prosper under her leadership, and I look forward to continuing to work with her and the rest of the Board of Directors on advancing our mission.

There's a long list of people that have contributed to making this meeting happen, but none more important than that of my fellow ASCCT members that served on this year's meeting organizing committee. Many thanks go out to this group, as well as the Board of Directors for planning this year's meeting. And of course, thank you for coming to share your work and get involved in the Society!

The ASCCT continues to be a platform to exchange ideas among regulatory and research scientists from both the computational and cellular sides of predictive toxicology. Last year, I charged you all to be sure the year ahead sees our members continuing to foster open dialog between industry, academic, advocacy, and regulatory scientists; include the participation of young scientists to promote their contributions to the field; and strengthen cooperation between government and industry scientists and professionals. I'm pretty sure this year's program suggests that we collectively nailed it…

Have a great meeting!
Dave

Program Committee

David Allen
Elizabeth Baker
Sudin Bhattachrya
Marie Fortin
Jack Fowle
Thomas Hartung
Esther Haugbrooks
Erin Hill
Shaun McCullough
Mirriam Mossoba
Bakhtiyor Rasulev
Kristie Sullivan
Lena Smirnova
Mark Williams
Hao Zhu

Board of Directors

David Allen, President
Ellen Berg
Gertrude-Emilia Costin
Marie Fortin
Jack Fowle
Thomas Hartung
Erin Hill, Treasurer
Shaun McCullough
Kristie Sullivan, Secretary

2018 ASCCT Agenda

7th Annual Meeting of the ASCCT
Predictive Toxicology: Strategies for Implementing New Approaches

Tuesday, September 11, 2018

8:30 am	Welcome and Introduction *Erin Hill, Vice President, ASCCT*
8:40 am	Building Biological Bridges: Facilitating the Predictive Toxicology Paradigm *Brian Berridge, National Toxicology Program*
9:20 am	Predictive Toxicology for Regulatory Decisions: Implementing New Approaches at FDA *Suzanne Fitzpatrick, Food and Drug Administration*
9:50 am	Microphysiological Systems - The Opportunities and the Challenges *Lorna Ewart, AstraZeneca*
10:20 am	**Break**
10:30 am	Computational Model of Chemical Transport in PDMS-Based Organ-On-Chip Microsystems for Toxicity Studies *K. Tasneem, Vanderbilt University*
10:50 am	3D Liver Microtissues Bridge Applications from Drug-Induced Liver Injury (DILI) to Low-Clearance in ADME Seamlessly *Jan Lichtenberg, InSpheroAG*
11:10 am	Using Human Primary Endothelial Progenitor Cells for Toxicological Risk Assessment *Daria Filonova, Creative Scientist*
11:30 am	Non-Animal Approaches in the Complex Regulatory Environment of Newly Deemed Tobacco Products: Possibilities, Limitations, and Looking Forward *Phil Yeager, Center for Tobacco Products, FDA*
12:00 pm	Using Alternative Approaches for the Assessment of Next Generation Nicotine and Tobacco Products *Marianna Gaca, British American Tobacco*
12:30 pm	**Lunch & Poster Session**

2:00 pm	Qualification of Non-Animal Methods for Biocompatibility Assessment of Medical Devices: Use of Medical Device Development Tools (MDDTs) *Jennifer Goode, Center for Devices & Radiological Health, FDA*
2:30 pm	Development of a Non-Animal Method for Biocompatibility Assessment of Personal Lubricants Under the US FDA's MDDT Program *Emilia Costin, Institute for In Vitro Sciences*
3:00 pm	Maximizing the Impact of Tissue Chip Technologies with *In Vitro In Vivo* Translation *Murat Cirit, Massachusetts Institute of Technology*
3:30 pm	Collaboration to Develop Predictive Models for Acute Oral Systemic Toxicity *Kamel Mansouri, Integrated Laboratory Systems, Inc./ NTP Interagency Center for the Evaluation of Alternative Toxicological Methods*
3:50 pm	Population Lifecourse Exposure to Health Effects Model (PLETHEM) - An Open Source R Package that Unifies Exposure and Pharmacokinetic Tools *Salil Pendse, ScitoVation*
4:10 pm	Developing Mechanism-Based Animal Toxicity Models: A Chemocentric Approach Using Big Data *Daniel Russo, Rutgers University*
4:30 pm	**Break**
4:45 pm	The Re-Envisioning of the National Library of Medicine's Toxicology and Environmental Health Resources *Pertti (Bert) Hakkinen, National Library of Medicine*
5:00 pm	Panel Discussion: Public-Private Partnerships as a Mechanism for Implementing New Approaches *Moderator: Dave Allen, President, ASCCT* *Panelists:* Lorna Ewart, AstraZeneca Brian Berridge, NTP Paul Brown, FDA CDER Phil Yaeger, FDA CTP Jessica Palmer, Stemina Biomarker Discovery
6:00 pm	**Reception & Awards Ceremony** Sponsored by Alternatives Research and Development Foundation

ASCCT

American Society for Cellular and Computational Toxicology

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:

A 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

www.ascctox.org info@ascctox.org

Table of Contents

Invited Abstracts	09
Oral Abstracts	17
Poster Abstracts	25
Awards	61

ASCCT 2018
Invited Abstracts

Building Biological Bridges: Facilitating the Predictive Toxicology Paradigm

B. R. Berridge

In 2004, the National Toxicology Program articulated an aspirational vision shared by many in the toxicology community to "support the evolution of toxicology from a predominately observational science …to a predominately predictive science…". Despite a clear need to make that shift and significant advances in our ability to generate high volumes of cellular mechanistic data, decision-making confidence is still largely entrenched within the phenotypic outcomes of animal studies. It's possible that we've attempted a bridge too far by attempting to extrapolate outcomes from biologically simple systems to increasingly complex human contexts. This challenge encompasses the human relevance of simple modeling systems, relating biological events at the cellular level to events at the organism level, connecting mechanisms to phenotypes and projecting long term outcomes from short term exposures. This presentation will explore this fundamental challenge, identify a discrete gap in our approaches and discuss elements of a strategy to enable the evolution we need to meet contemporary challenges and expectations.

Associate Director, National Toxicology Program

Predictive Toxicology for Regulatory Decisions: Implementing New Approaches at FDA

S. Fitzpatrick

Toxicology testing plays a pivotal role in ensuring the safety of FDA-regulated products. During the development and evaluation of almost all FDA-regulated products, testing is performed on people or animals to identify any potential risk from chemical, physical, or biological agents. For a new testing method to be accepted for use in determining the safety of an FDA-regulated product, there must be sufficient convincing data to ensure that the method can be relied upon for both product development and regulatory decision-making. In 2016, FDA's Commissioner tasked the agency's Toxicology Working Group with developing a more efficient process for identifying and qualifying emerging predictive toxicology technologies. Established in 2015 and comprising senior FDA toxicologists across the agency, the Working Group has deep expertise in the various FDA product areas and knowledge of the differing legal authorities for evaluating safety and toxicity in those product areas. In December, 2017, the FDA announced its Predictive Toxicology Roadmap, a six-part framework for integrating predictive toxicology methods into safety and risk assessments. Because this is a high priority for the agency, FDA's Toxicology Working Group will be reporting yearly to FDA's Chief Scientist on progress made in this important effort. FDA is confident that the successful implementation of FDA's predictive toxicology roadmap and the continuing engagement of our diverse stakeholders will enable FDA to fulfill its regulatory mission today while preparing for the challenges of tomorrow.

U.S. Food and Drug Administration

Microphysiological Systems – the Opportunities and the Challenges

L. Ewart

Microphysiological systems (best recognized as 'organ chips') are a rapidly developing *in vitro* technology that provides unique opportunities to model the complexity of *in vivo* biology and to explore aspects of biology that are not currently possible *in vitro*. The growing debate about the translational value of animal data together with the challenging rate of drug development attrition for both efficacy and safety has prompted excitement for the potential of clinically relevant microphysiological systems. Attaining the full promise and impact of this innovation will take a very deliberate process of evolution and requires significant partnership across engineers, biologists, regulators and policy makers. This presentation will introduce the hype and hope of microphysiological systems as well as outlining the framework used at AstraZeneca to select the context of use for microphysiological systems. It will also exemplify the type of data that can be achieved from such systems in drug development while highlighting some of the outstanding challenges that need to be overcome to realise the true value of these models.

Astra Zeneca

Using Alternative Approaches for the Assessment of Next Generation Nicotine and Tobacco Products

M. Gaça

There has been a significant increase in the number of smokers using next generation tobacco and nicotine products (NGPs), including e-cigarettes. The growing popularity amongst consumers and the pending regulations for the product category demonstrates the importance of e-cigarettes as an emerging scientific field. The use of *in vitro* tests developed to model key endpoints associated with smoking-related disease can provide valuable insights into the disease mechanisms associated with tobacco use and may be suitable for the assessment of NGPs.

We will present the challenges and opportunities offered using *in vitro* approaches, integrating adverse outcome pathways and next generation technology as part of a product assessment framework, evaluating the availability of an *in vitro* toolbox, with a focus on inhalation toxicology. A number of assays and endpoints will be explored including the use of classical *in vitro* toxicological assays specifically measuring mutagenicity and cytotoxicity, and showing greatly reduced responses from NGPs relative to cigarettes. Human cellular based *in vitro* assays will be presented including the use of continuous cell lines to more complex physiological organotypic reconstituted tissue systems, integrating adverse outcome pathways and next generation technology. These studies will demonstrate how *in vitro* assays can provide data to support the risk assessment of NGPs as part of a larger weight of evidence.

British American Tobacco

Development of a Non-Animal Method for Biocompatibility Assessment of Personal Lubricants Under the US FDA's MDDT Program

G.E. Costin[1], E. Hill[1], J. Brown[2]

In 2017 the US FDA launched its Predictive Toxicology Roadmap which calls for the optimization of non-animal methods for the risk assessment and safety evaluation of drugs, consumer products and medical devices. Integrated in this wide modernization plan is the Medical Devices Development Tools (MDDT) Program which recently accepted our proposal of an *in vitro* testing approach based on human reconstructed tissue model(s) that could replace the Rabbit Vaginal Irritation (RVI) Test. This initiative addresses the need for manufacturers of personal lubricants (considered Class II medical devices in the United States) to receive pre-market clearance from the US FDA Center for Devices and Radiological Health (CDRH) based on a recommended biocompatibility testing battery that includes the RVI Test. Thus, IIVS created an Industry Consortium, comprised of manufacturers of personal lubricants/vaginal moisturizers and companies interested in the advancement of animal alternatives, which works collaboratively with stakeholders and the US FDA to develop an *in vitro* testing approach that could be used in place of the RVI in pre-market submissions. Our validation program will focus on personal lubricants and vaginal moisturizers with diverse chemical and physical properties (e.g., formulation, viscosity, pH, osmolality) in their final, undiluted, form. Paired *in vivo-in vitro* data for vaginal irritation generated using commercially available human reconstructed vaginal tissue model(s) will be analyzed against existing *in vivo* RVI data to develop a prediction model for the safety assessment of these products. Accepted as a Nonclinical Assessment Model (NAM) in the Incubator Phase, our validation program is currently being reviewed for consideration to advance to the Pre-Qualification state. This type of collaborative work and partnership between industry, regulators and other organizations supporting non-animal testing methodologies is the solution for implementation of modern predictive toxicology platforms that can support regulatory decisions.

[1]Institute for In Vitro Sciences, Inc. (IIVS), [2]PETA International Science Consortium

Maximizing the Impact of Tissue Chip Technologies with *In Vitro In Vivo* Translation (IVIVT)

M. Cirit

A large percentage of drug candidates fail at the clinical trial stage due to a lack of efficacy and unacceptable toxicity, primarily because the *in vitro* cell culture models and *in vivo* animal models commonly used in preclinical studies provide limited information about how a drug will affect human physiology. The need for more physiologically relevant *in vitro* systems for preclinical efficacy and toxicity testing has led to a major effort to develop "Microphysiological Systems (MPS)", aka tissue chips (TC), based on engineered human tissue constructs. Translational Center of Tissue Translational Center of Tissue Chip Technologies (TC2T) has been established to bridge between academic research and development and industrial application of MPS technologies.

TC2T takes a holistic and mechanistic approach—based on quantitative systems pharmacology (QSP)— to achieve unbiased characterization of these complex systems and translation of experimental insights to clinical outcomes. QSP models for specific applications informed with MPS results and human physiology can be used to translate *in vitro* results from MPSs in to the *in vivo* human context

QSP models are initially developed based on an array of biological and (patho) physiological data as well as information about target and drug characteristics. Then, quantitative information derived from MPS experimentation provides values or ranges of specific parameters to the models. MPS results might relate specifically to drug activity or might provide biology-specific parameters with which to define virtual patient biology for simulations of drug activity derived from other sources. Simulation and analysis using MPS inputs are then possible to predict aspects of drug efficacy, toxicity, DMPK, etc., for virtual patients (individuals, populations, subpopulations) accounting for knowledge gained from MPSs.

Massachusetts Institute of Technology

ASCCT 2018
Oral Abstracts

Computational Model of Chemical Transport in PDMS-Based Organ-On-Chip Microsystems for Toxicity Studies

K. Tasneem[1], A. Auner [2], D. Markov [3,4], L. J. McCawley[3,4], M. S. Hutson[2,4,5]

kazi.m.tasneem@vanderbilt.edu

Organ-on-chip microsystems are being used to evaluate chemical responses to human cells cultured in 3D environments. These microsystems are often fabricated from polydimethylsiloxane (PDMS), which has high affinity for small hydrophobic molecule creating undesirable change in dose response curves and timing of toxic exposure to cells. We modeled the chemical adsorption onto PDMS surfaces in such system using computational fluid dynamics. The goal is to predict the cultured cells' actual time-dependent toxic exposure within a PDMS fabricated microsystem. We established quantitative relationships for chemical adsorption onto PDMS surfaces through fitting spectroscopic data to microscale model of chemical binding kinetics with PDMS and extracted time-dependent adsorption coefficients. Experimental rate constants were used to model adsorption due to continuous and bolus exposure of chemicals within microsystem. Model predicted that timing is critical for delivery of chemicals that reversibly bind to PDMS in order to avoid over- or under-dosing cells. For such chemicals, a bolus dose at the inlet may translate into an extended exposure for cells in the device due to delayed release from PDMS surfaces. In addition, for chemicals with strong affinity to PDMS, the actual exposure may be an order of magnitude less than the nominal inlet concentration. This computational model is, therefore, greatly relevant to the accuracy of chemical toxicity and will significantly facilitate the design of chemical dosing strategy in the toxicity studies in microfluidic platforms, leading to a potential outcome in the form of a more effective and safe assessment of potential toxic chemicals.

[1]Department of Chemical and Biomolecular Engineering, Vanderbilt University, [2]Department of Physics and Astronomy, Vanderbilt University, [3]Department of Biomedical Engineering, Vanderbilt University, [4]Vanderbilt Institute for Integrative Biosystems Research and Education, Vanderbilt University, [5]Department of Biological Sciences, Vanderbilt University

3D Liver Microtissues Bridge Applications from Drug-Induced Liver Injury (DILI) to Low-Clearance in ADME Seamlessly

R. Kostadinaova, O. Frey, B. Manning, J. Lichtenberg

jan.lichtenberg@insphero.com

Primary hepatocytes in 2D culture (PHH) are widely used for prediction DILI. However their predictivity is limited due to rapid de-differentiation, which limits testing to acute toxicity. 3D InSight™ Human Liver Microtissues (hLiMT) consist of primary multi-donor hepatocytes and Kupffer cells and have shown liver specific function and metabolic cytochrome activity over five weeks in culture. The utility of the 3D hLiMT for short- and long-term drug toxicity was investigated. The assessment of the toxicity using ATP as a cell viability marker has been performed of 110 marketed drugs with known DILI potential on 2D PHH and 3D hLiMT. The hLiMT, exhibited a more than 2-fold increased sensitivity for detection of DILI compounds. The results demonstrated that 3D hLiMT are a suitable model for assessment of DILI.

In a second step, 3D hLiMT were loaded into a microfluidic, microphysiological chip device (Akura™ Flow) to enable constant perifusion combined with an increased cell-to-medium ratio. In this configuration, 3D hLiMTs have been used to quantify clearing rates of low-clearance compounds, including Tolbutamide and Warfarin.

Both experiments illustrate how the same assay-ready 3D hiLMT model can be used in static applications using 96w or 384w plates for DILI assessment, followed by clearance quantification under static conditions and finally low-clearance quantification under flow. As the model, cell sources and culture medium remain essentially the same, a seamless transition between the different phases of safety assessment become finally possible.

InSphero AG

Using Human Primary Endothelial Progenitor Cells for Toxicological Risk Assessment

D. Filonova[1], R. Tice[1], C. Grotegut[2], R. Luo[3], D. Il'yasova[3], A. Kinev[1]

avkinev@gmail.com

Human blood contains several rare populations of progenitor cells, one of which is able to differentiate into endothelial cells and form blood vessels throughout an organism's lifespan. These cells, called late outgrowth endothelial progenitor cells or endothelial colony-forming cells (ECFCs), will form blood vessels *in vivo* and *in vitro*. Isolated ECFCs can be clonally expanded into large numbers of viable cells (>109), which is important for developing ECFC-based high throughput assays suitable for the screening of chemicals for their ability to affect endothelial cell function. Endothelial dysfunction is a hallmark of many circulatory disorders, including cardiovascular disease and diabetes. In addition, endothelial dysfunction in embryos can result in reduced angiogenesis, which could lead to either developmental abnormalities or long-term functional deficiencies.

We have established a library of cord- and peripheral blood derived ECFCs and have developed a series of ECFC-based assays with a goal of reducing animal use in toxicological studies, while increasing the relevance of the results to humans. Initially, we conducted a simple viability assay using early passage proliferating ECFCs to assess individual variability in response to several toxicants. For some toxicants (e.g., Tributyltin, arsenic), the ECFCs from different donors exhibited the same sensitivity but exhibited donor-specific differences in response to other toxicants (e.g., Menadione). In addition, using the donor-specific ECFCs, we have developed a high throughput/high content anti-angiogenesis assay and have characterized its utility for the screening of chemicals that can potentially affect human angiogenesis and, therefore, exert developmental toxicity.

[1]Creative Scientist, Inc., [2]Duke University, [3]Georgia State University

Collaboration to Develop Predictive Models for Acute Oral Systemic Toxicity

A. Karmaus[1], K. Mansouri[1], J. Fitzpatrick[2], G. Patlewicz[2], D. Allen[1], W. Casey[3], N. Kleinstreuer[3]

akarmaus@ils-inc.com

Acute oral systemic toxicity testing is required by regulatory agencies world-wide with acute toxicity tests representing the highest cumulative animal use across chemical sectors. One of ICCVAM's priorities is to develop alternative methods for acute toxicity tests, beginning with the acute oral systemic toxicity assay. To support this goal, NICEATM organized an international collaborative effort to develop *in silico* models for the acute oral systemic toxicity assay that would address ICCVAM agency regulatory needs. NICEATM and EPA's NCCT compiled rat acute oral lethality data (LD50 values) and associated structure information for 11,992 chemicals to facilitate developing predictive *in silico* models of acute oral systemic toxicity. This data set was used by collaborators for predictive modeling with varying approaches, applicability domains, strengths, and limitations. In total, the consortium of 35 groups delivered 139 models. These models were combined to establish consensus predictions for five different endpoints covering binary, categorical, and continuous values. The consensus predictions leverage each model's strengths and overcome the limitations of any individual approach. A summary of the compiled LD50 dataset as well as details regarding the predictive modeling will be presented. This unique approach to developing alternatives to animal testing involved initiating work with a definition of needs set forth by regulatory agencies, involving diverse stakeholders at every step, and integrating collective expertise from the international modeling community. US federal funds from NIEHS/NIH/HHS Contract HHSN273201500010C supported this work; the views expressed are those of the authors and do not necessarily reflect US EPA views or policy.

[1]ILS, [2]EPA/ORD/NCCT, [3]NIH/NIEHS/DNTP/NICEATM

Population Lifecourse Exposure to Health Effects Model (PLETHEM): An Open Source R Package That Unifies Exposure and Pharmacokinetic Tools

S. N. Pendse[1], C. Nicolas[1], A. Y. Efremenko[1], C. Housand[1], P. McMullen[1], M. Yoon[1,2], H. J. Clewell 3rd[1,3]

spendse@scitovation.com

The EPA Office of Research and Development's 2003 framework for computational toxicology emphasized the need for computational methods to bridge the source-to-outcome continuum. This goal can be achieved by linking exposure estimation methods, physiologically based pharmacokinetic (PBPK) modeling, and computational systems biology pathway modeling tools into a standardized framework. To that end, we have developed the Population Lifecourse Exposure To Health Effects Model (PLETHEM) suite, a modular open source modeling platform that provides users the ability to create, share, and audit PBPK models and connect them to existing exposure tools. The platform consists of a database of chemicals, QSAR models, life-course equations, and metabolism parameters needed to perform PBPK modeling, an R- based engine to perform model simulations, and an interactive user interface to define and select parameter sets for the models. PLETHEM includes the ability to run Monte Carlo analyses to investigate population variance and a set of life course equations to investigate life stage based sensitivities. The PLETHEM database also incorporates ontogeny profiles for key metabolic enzymes that can be used to calculate *in-vivo* metabolic clearance using measured *in vitro* clearance. PLETHEM is currently available as an R package though open source repositories. This research was funded by the American Chemistry Council and is being conducted under a Memorandum of Understanding with the USEPA.

[1]ScitoVation LLC, [2]ToxStrategies Inc, [3]Ramboll Environ

Developing Mechanism-Based Animal Toxicity Models: A Chemocentric Approach Using Big Data

D. P. Russo[1], J. Strickland[2], A. Karmaus[2], W. Wang[1], S. Shende[1, 3], T. Hartung[4, 5], L. M. Aleksunes[6], H. Zhu[1, 7]

danrusso@scarletmail.rutgers.edu

High-throughput *in vitro* bioassays show potential as alternatives to animal models for toxicity testing. Big data resources, e.g. PubChem, are updated continuously with new *in vitro* bioassay data, resulting in a massive amount of ever-changing public data. However, incorporating *in vitro* bioassays from these resources into chemical toxicity evaluations requires significant data curation and analysis based on knowledge of relevant toxicity mechanisms. In this work, we aimed to develop a computational method to automatically extract useful bioassay data from PubChem and assess its ability to predict animal toxicity using read-across. To achieve this, a database containing 7,385 compounds with diverse rat acute oral toxicity data was searched against PubChem to establish *in vitro* bioprofiles. Using a novel subspace clustering algorithm, bioassays were grouped together based upon chemical substructures identified as significant to bioassay activity. Several bioassay groups showed high predictivity for animal acute oral toxicity using read-across through a cross-validation process (positive prediction rates range from 62%-100%). The predictivity of these models were further validated using a set of over 600 new compounds. Incorporating individual clusters into a consensus model, chemical toxicants in the validation set were prioritized (positive prediction rate equal to 76%). In addition to high predicitivity, chemical fragment – *in vitro* – *in vivo* relationships can be highlighted in bioassay clusters to illustrate new animal toxicity mechanisms. This data-driven profiling strategy meets the urgent needs of computational toxicology in the big data era and can be extended to develop predictive models for other complex toxicity endpoints.

[1]Center for Computational and Integrative Biology, Rutgers University, [2]Integrated Laboratory Systems, Inc., [3]Department of Computer Science, Rutgers University, [4]Johns Hopkins Bloomberg School of Public Health, Center for Alternatives to Animal Testing (CAAT), [5]University of Konstanz, CAAT-Europe, [6]Department of Pharmacology and Toxicology, Ernest Mario School of Pharmacy, [7]Department of Chemistry, Rutgers University

ASCCT 2018

Poster Abstracts

Cost-effective Techniques for 3D Culture of Heterotypic Tumor Models

M.A. Akbarsha[1, 2], A. Balaji[3,4], S. Shanmugaapriya[4], L. Paasonen[5]

akbarbdu@gmail.com

Three dimensional (3D) co-cultures of various cell types to simulate tumors bridge the gap between 2D cultures and animal models / *in vivo* human patients, enable a better understanding of the molecular and cellular mechanisms, and facilitate development newer drugs for cancer in a manner better than monolayer cell culture systems. One of the main requirements in generating 3D *in vitro* systems is the scaffold which supplies nutrients and mimics extracellular matrix (ECM) conditions. Hydrogels fabricated from biological or synthetic materials serve as suitable scaffolds to provide the 3D- microenvironment and, hence, attempts are in progress to develop novel and cost-effective scaffolds for 3D culture of cancer cells. Thus, in our study, we used nanofibrillar GrowDex as well as hen's (Gallus domesticus) egg white to generate 3D multicellular (heterotypic) tumor spheroids (MTS). The epithelioid tumor cell lines, the small cell lung carcinoma A549 and triple negative breast cancer MDA-MB-231, were cultured in both 3D microenvironment and conventional 2D cell culture system. The results revealed superiority of 3D model over monolayer cell culture in terms of proliferation efficiency, cytoskeletal organization of cells within spheroids and creation of tumor micro-environment. Co-culturing of these two different epithelial cells with NIH/3T3 fibroblast cell influences the size and shape of the spheroid. Distribution and interaction patterns of fibroblast and epithelial cells in co-culture spheroids were also observed. Our data suggest that ideal, non-toxic and tunable GrowDex nanocellulose and egg white hydrogels facilitate culture of different cell types and to produce heterotypic 3D MTS under *in vitro* condition and, hence, further studies are in progress to characterize the tumor microenvironment in order to develop this as a suitable model that mimics the animal model / *in vivo* human.

[1]National College (Autonomous), [2]Mahatma Gandhi – Doerenkemp Center for Alternatives, Bharathidasan University, [3]National Center for Alternatives to Animal Experiments, Bharathidasan University, [4]Department of Biomedical Science, Bharathidasan University, [5]GrowDex® Biomedicals, UPM Tukholmankatu 8, Biomedicum

Frank R. Lautenburg Chemical Safety for the 21st Century Act: Potentials in Small Invertebrate Model Organisms with Special Reference to Hydra

M.A. Akbarsha[1, 2], A. Murugadas[3,4], M. Zeeshan[2,3], F. P. Gruber[5]

akbarbdu@gmail.com

Animal testing of chemical entities greatly relies on mammalian model organisms. In the light of emergence of 3Rs from perspectives of ethical considerations as well as species differences, ecotoxicity testing of water bodies is extended to the lower vertebrate model organisms. The Tox-21c recommends the use of lower vertebrate animal models for eco-toxicological investigations of chemical entities. The more recent Frank R. Lautenburg Chemical Safety for the 21st Century Act requires a moratorium on the use of vertebrate organisms in toxicity testing, providing scope for invertebrate organisms and *in silico* approaches in the place of vertebrate organisms. In the look-out for convenient and appropriate invertebrate model organisms which belong to the lower sentient level and which under ambient conditions cannot be depleted, we adopted Hydra, a freshwater cnidarian, as a model organism for risk assessment of environmental chemicals which pollute the aquatic bodies. Though simple in organization and biology, it is much complex compared to cultured cells, which make it an amenable organism for eco-toxicity testing. Besides, Hydra offers advantages such as easy to culture, reproduces fast, cost-effective and highly sensitivity to inorganic pollutants. We took to advantage the whole genome sequencing of Hydra which revealed conserved sequences and signaling pathways. The data to be presented here will demonstrate the versatile behavior of Hydra and suitability of Hydra for toxicity testing of nanomaterials as well as their bulk counterparts. Acute and chronic studies performed with sub-lethal doses of nanoparticles revealed physiological, developmental and behavioral responses in Hydra. Metagenome sequencing revealed influence of nanoparticles on commensal bacterial population and the factors that contribute to maintain the bacterial community structure. Molecular studies uncovered the underlying mechanism of toxicity in a manner very precise. TEM analysis revealed sub-cellular alterations and accumulation of nanoparticles within the cells of Hydra. The data to be presented will substantiate the use of Hydra as a convenient model organism for aquatic toxicity testing.

[1]National College (Autonomous), [2]Mahatma Gandhi-Doerenkamp Center (MGDC) for Alternatives, [3]Department of Environmental Biotechnology, Bharathidasan University, [4]National Centre for Alternatives to Animal Experiments, Bharathidasan University, [5]Doerenkamp-Zbinden Foundation

Public-Private Partnerships to Advance *In Vitro* Eye Irritation Testing Methods and Approaches

D. Allen[1], N. Choksi[1], S. Gehen[2], W. Casey[3] A. Clippinger[4]

dallen@ils-inc.com

The 16 U.S. government agencies that comprise the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recently released a roadmap and strategy to expedite the development, use, and regulatory acceptance of new approach methodologies that provide more human-relevant information than *in vivo* methods currently used in human health assessments. A central theme to realizing success is the formation of public-private partnerships that allow cross-sector communication and cooperation among federal agencies and the private sector. These provide a means for sharing knowledge, experience, and data to most efficiently advance test method development and evaluation. Such partnerships are being implemented to advance alternatives for eye irritation testing for chemicals and formulations. While multiple *in vitro* methods have been adopted to characterize the eye irritation potential of a wide range of substances, a complete replacement for the *in vivo* test method has yet to be globally accepted. Global acceptance is further confounded by the differences in requirements for hazard classification and labeling between U.S. and international regulatory authorities. As a result, international stakeholders from government, industry, academia, and non-governmental organizations are collaborating on the development of approaches for eye irritation testing that can be applied to specific types of substances, such as agrochemical formulations. We report on the international efforts to replace animal use for eye irritation testing, and provide a model for multi-stakeholder collaborations that could be applied to other areas. This was funded with U.S. federal funds from the NIEHS/NIH/HHS under Contract HHSN273201500010C.

[1]ILS, [2]Corteva Agriscience, [3]NIH/NIEHS/DNTP/NICEATM, [4]PETA International Science Consortium Ltd.

The Nonclinical Innovation and Patient Safety Initiative (NIPSI): Supporting Human-Based Nonclinical Testing Through Regulatory, Policy, Educational and Training Advances

E. Baker

ebaker@pcrm.org

A growing number of drug development stakeholders agree that integration of modern and state-of-the-art human-based nonclinical approaches for assessing drug safety, efficacy and disease modeling will improve drug development. Notably, the Food and Drug Administration (FDA) and Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recently launched roadmaps describing plans to do so. The Nonclinical Innovation and Patient Safety Initiative (NIPSI) formed in 2017 to drive stakeholder collaboration on projects to improve the human-relevance of nonclinical testing. Stakeholders initially met at a full-day NIPSI roundtable in January 2017 in Washington, D.C., then an evolving group met during ancillary meetings of the Society of Toxicology Annual Meetings in March 2017 and March 2018. This presentation outlines NIPSI recommendations for advancing the uptake of human-relevant nonclinical approaches. One project aims to change current FDA regulations that require animal data, to reflect FDA's discretion to accept human-based nonclinical approaches in investigational new drug applications (IND) and new drug applications (NDA). This is necessary for ensuring FDA regulations at minimum keep pace with innovative science. Another project involves lobbying the United States Congress to increase funding allocated for human-based science. The presentation will also include results from a recent review of NDAs that was conducted to determine if the FDA has received data from organ chips in NDAs.

Physicians Committee for Responsible Medicine

User-Friendly Toxicology Tools from ICE Workflows

S. Bell[1], J. Phillips[2], N. Cariello[1], P. Ceger[1], X. Chang[1], F. Hermes[1], K. Mansouri[1], R. Rai[1], D. Allen[1], W. Casey[3], N. Kleinstreuer[3]

sbell@ils-inc.com

Developing and evaluating new approaches to chemical safety testing require quality data and supporting information such as chemical properties and key modeling parameters. These activities also require tools that can put assay data into the appropriate biological context and determine whether a given dataset is appropriate for evaluating a method of interest. To address these needs, the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) developed the Integrated Chemical Environment (ICE) to house high-quality, curated *in vivo* data along with *in vitro* and *in silico* values for a range of endpoints. In this presentation, we announce the launch of ICE Workflows, which support chemical characterization, assist in data analysis and model building, and enable direct comparisons between *in vitro* assay data and relevant *in vivo* endpoints. ICE Workflows are a series of interactive tools that allow users to use ICE data to characterize the chemical space covered by their chemical lists, perform basic machine learning functionality, and generate *in vitro* to *in vivo* extrapolation predictions. Leveraging the ICE ontology, users can easily identify assays and data points within ICE that are relevant to their toxicological outcome of interest. This facilitates exploration of data and hypothesis generation for users without a strong background in computational toxicology. Future functionalities to be added will also be discussed, including input of user-supplied data. ICE was funded with U.S. federal funds from the NIEHS/NIH/HHS under Contract HHSN273201500010C.

[1]ILS, [2]Sciome LLC, [3]NIH/NIEHS/DNTP/NICEATM

Integrating Genomics and Epigenomics Into Predictive Toxicology of the Aryl Hydrocarbon Receptor

S. Bhattacharya

sbhattac@msu.edu

In the canonical model of gene regulation by the ligand-inducible transcription factor aryl hydrocarbon receptor (AHR), the AHR forms a heterodimer with the related nuclear protein aryl hydrocarbon nuclear translocator (ARNT) and binds cognate recognition sites (AHR response elements, AHREs) in the promoter regions of target genes harboring the 5-bp core motif 5'-GCGTG-3'. However, the vast majority of 5'-GCGTG-3' sequences in the genome do not bind the AHR upon ligand activation. Conversely, a minority of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)-induced genes show AHR binding to AHREs in their proximal promoters. What then are the determinants of AHR binding genome-wide, and the mechanisms by which the AHR regulates target genes?

We have used a combination of published ChIP-Seq data, bioinformatic analysis, and machine learning models to identify sequences flanking the 5-bp core motif that distinguish bound from unbound AHREs. In addition, we found considerable overlap genome-wide between AHR binding and binding by the CCCTC-binding factor (CTCF) and cohesin protein complex, suggesting a significant role for DNA looping and long-range chromatin interactions in AHR-mediated gene regulation. We present a predictive model of proximal and distal gene regulation by the AHR that can be validated by a combination of long-range chromatin interaction assays and targeted epigenome editing (CRISPR interference).

This work provides a general framework to map gene regulatory networks mediated by nuclear receptors and other ligand-activated transcription factors, and develop more mechanistic predictive models of toxicity associated with activation of these factors.

Departments of Biomedical Engineering and Pharmacology & Toxicology, Center for Research on Ingredient Safety, Institute for Quantitative Health Science and Engineering, Michigan State University

Development of Free and Open-Source QSAR Tools for Predicting pKa

N. Cariello[1], K. Mansouri[2], A. Korotcov[2], V. Tkachenko[2], W. Casey[3], A.J. Williams[4]

ncariello@ils-inc.com

The logarithmic dissociation constant, pKa, provides information about the ionization state of a chemical, which affects its lipophilicity, solubility, protein binding, and ability to cross the plasma membrane of a cell. These properties govern pharmacokinetic parameters such as absorption, distribution, metabolism, excretion, and toxicity. Therefore, accurate pKa predictions are critical for the assessment of chemical toxicity and biological activity. Predictions of pKa can be made using empirically based methods such as quantitative structure–activity relationships (QSARs) and quantum mechanical approaches such as density functional theory (DFT). A number of commercial pKa prediction software tools are available but very little exists in terms of open data sets and open prediction models. Predicting pKa is particularly challenging due to the lack of high-quality publicly available experimental data restricting the resultant QSAR models to specific chemical domains. The aim of this work was to provide free and open-source pKa predictors using a large publicly available experimental pKa dataset obtained from DataWarrior (www.openmolecules.org). Chemical structures were standardized for model fitting and validation. Three different machine learning algorithms, support vector machines, extreme gradient boosting and deep neural networks were used to build models using continuous descriptors and binary fingerprints generated by PaDEL. The best performing models for each algorithm were benchmarked using predictions from two commercial tools, ACD/Labs and Chemaxon, on an untested list of chemicals. This comparison showed varying degrees of concordance among the models, including between the proprietary tools. This was funded with U.S. federal funds from the NIEHS/NIH/HHS under Contract HHSN273201500010C.

[1]ILS, [2]Science Data Software, LLC, [3]NIH/NIEHS/DNTP/NICEATM, [4]EPA/ORD/NCCT

An Open Source IVIVE Workflow Integrating *In Vitro* Data, QSAR Models and Reverse Dosimetry

X. Chang[1], S. Bell[1], R. Rai[1], J. Phillips[2], K. Mansouri[1], D. Allen[1], W. Casey[3], N. Kleinstreuer[3]

xchang@ils-inc.com

A critical challenge to implementing non-animal approaches for chemical safety testing is linking *in vitro* assay results to potential *in vivo* effects. To address this challenge, we have developed an *in vitro* to *in vivo* extrapolation (IVIVE) workflow incorporating *in vitro* data, QSAR models, and reverse dosimetry with a population-based one-compartment pharmacokinetic (PK) model. The resulting IVIVE workflow is available through NICEATM's Integrated Chemical Environment (ICE) web resource. It can be used within ICE as an interactive online tool or can be downloaded to run locally. The workflow allows estimation of steady-state blood concentration (Css) across a simulated population following a given dose. It also allows prediction of the external dose leading to a Css equivalent to effective concentration in user-selected *in vitro* assays. Where data are available, the predicted external dose can be compared to *in vivo* experimental doses for the same chemical. This comparison can be used to establish confidence in the model's performance when generating predictions for structurally related compounds lacking *in vivo* data. Users can supply their own PK parameters (e.g., fraction unbound to plasma protein, hepatic clearance), or parameters can be predicted based on chemical structures using OPERA's QSAR models. Using the estrogen receptor pathway as an example, this presentation will demonstrate how the IVIVE workflow can be used to evaluate the correlation between *in vitro* and *in vivo* dosimetry for toxicologically relevant endpoints. This was funded with U.S. federal funds from the NIEHS/NIH/HHS under Contract HHSN273201500010C.

[1]ILS, [2]Sciome LLC, [3]NIH/NIEHS/DNTP/NICEATM

Human Cell Line Activation Test (h-CLAT): Pitfalls and Considerations

G.L. DeGeorge, P. Vij, D. Sergeyev, B.J. Varsho

mbweb@mbresearch.com

Skin sensitization resulting from exposure to a chemical is an important concern. The h-CLAT was developed based on the role of Langerhans cells (LC) activation during the induction phase of skin sensitization and uses THP-1 cell line as LC surrogate. This *in vitro* test measures the augmentation of CD86 and CD54 expression in THP-1 cells (a human monocytic leukemia cell line) following a 24-hour exposure to a test substance (TS). Herein we address some pitfalls, issues and remediation in the h-CLAT procedure. The reactivity check, performed two weeks after thawing a new batch of cells, requires the viability of vehicle and Lactic Acid (LA) treated cells â‰¥90%. Both 2, 4-dinitrochlorobenzene (DNCB) and Nickel Sulfate (NiSO4) should produce a positive response in CD86 and CD54 expression, and LA should produce negative response. Otherwise, remedial steps should be taken, including thawing a new batch of cells. Proactive measures should be made to bank frozen cell stocks at various passages. An appropriate solvent (saline or DMSO) should completely dissolve the TS. If not, we present alternative vehicles (chosen with scientific justification) to broaden the applicability domain. A reliable CV75 should be derived from two independent screens. If it is difficult to attain, we present options for consideration. FACS buffer and 1% globulin preparation should be at 4Â°C. High Maintenance of the flow cytometer is essential for reliable data acquisition. In addition, historical data on the doubling time and passage number should be maintained and monitored.

MB Research Laboratories

Human *In Vitro* Models for Respiratory Toxicology: Evaluation of Goblet Cell Hyperplasia, Mucus Production, and Ciliary Beating Assays

S. Frentzel[1], M. Aragon[2], J. Hoeng[1], S. Ito[3], S. Ishikawa[3], J. Budde[4], A. Maione[5], P. Hayden[5], W. Fields[6], B. Keyser[6], L. Haswell[7], D. Azzopardi[7], and H. Behrsing[2]

bgilbert@iivs.org

Robust non-animal models and assays for pulmonary toxicology are required to make competent product development and risk assessments for new materials requiring toxicity testing. Three *in vitro* assays (goblet cell hyperplasia [GCH], ciliary beat frequency [CBF], and MUC5AC quantitation) were evaluated for performance and reproducibility. To assess these assays, 6 laboratories contributed data using a common protocol utilizing IL-13 as an inducer of adverse mucociliary-relevant tissue changes.

MatTek EpiAirway™ and Epithelix MucilAir™ 3D tissue models were used to evaluate endpoints using histology for GCH, software-based applications, Cilia FA and SAVA, for CBF, and ELISA assay for MUC5AC. Continuous 10 ng/mL IL-13 (GCH, MUC5AC) exposures or one hour 10 µM procaterol (CBF) exposures prior to day 7 and 14 time-points were included as positive controls. Quality control endpoints (e.g. adenylate kinase tissue content and trans-epithelial electrical resistance) were also evaluated.

Multi-fold increases (ranging from 2.6 to 33-fold, and 1.5 to 238-fold) in MUC5AC-stained goblet cells were measured in both tissue models after exposure with IL-13 after 7 and 14 days induction, respectively. For CBF, procaterol caused a significant increase, and IL-13 elicited a significant decrease as expected. However, the MUC5AC ELISA did not yield consistent results when frozen apical rinse samples were thawed and assayed.

These results suggest these non-animal test systems may provide consistent, human-relevant data corresponding to key events involved in respiratory disease. A streamlined protocol using these controls will be applied toward additional testing. These assays, utilized in a pragmatic manner with other *in vitro* assays have the potential to be included in a Reduced Risk Product assessment framework.

[1]PMI, [2]IIVS, Inc., [3]Japan Tobacco Inc., [4]ITL-Reemtsma Cigarettenfabriken GmbH, [5]MatTek, Inc., [6]British American Tobacco, [7]RAI Services Company

Use of Non-Animal Methods in Designing Inherently Safer Chemicals

S. C. Gehen, J. L. LaRocca, A. C. Lehman, K. J. Johnson

SCGehen@dow.com

Early-stage toxicologic assessments are often conducted as part of the product development cycle for new chemical entities across various sectors (e.g. pharmaceutical, agrochemical, industrial). While these assessments can take various forms, they serve the main purpose of identifying intrinsic hazard properties of candidate molecules to inform appropriate follow-up actions. In particular, computational and high-throughput *in vitro* toxicology methods can serve an important role in identifying potential liabilities and may also provide structural and mechanistic insights to aid in the design of molecules that eliminate the previously identified concerns. This presentation will highlight several approaches that have proven valuable in identifying favorable agrochemical development candidates. First, computational models in conjunction with *in vitro* tools, such as microsomal clearance assays, are used to predict and optimize mammalian bioavailability. For chemicals not intended as human drugs, the goal is to select analogs with lower potential bioavailability and systemic exposure. Next, screening against critical endpoints such as endocrine disruption is accomplished for example through conducting medium throughput *in vitro* estrogen and androgen receptor transcriptional activation assays. Additional focus on the steroidogenesis pathway can also be included. To further differentiate candidate analogs, *in vitro* cytotoxicity and transcriptomic assays with primary cells or cell lines are utilized to help define and characterize the intrinsic bioactivity and potency across a series of candidate compounds. Together, these approaches serve to enrich product development pipelines with candidate compounds with lower toxicity potential. Not only do the presented approaches rely exclusively on non-animal methods, their application is also expected to reduce animal use during development by minimizing potential for late-stage pipeline attrition and through more efficient use of animal testing based on greater a priori knowledge of compound properties.

Corteva Agriscience™, Agriculture Division of DowDuPont™

Evaluation of Cytotoxic Effects of Different Tobacco Product Preparations (TPPs) on Lung Cells

R.K. Gill[1], D. Guimet[2], C. Jin[2], S. Arimilli[3], H. Krebs[1], G.L. Prasad[1]

[1]RAI Services Company (RAIS), [2]ACEA Therapeutics Inc., [3]Winston-Salem

Agglomeration of Escherichia Coli with Positively Charged Nanoparticles Can Lead to Artifacts in a Standard Caenorhabditis elegans Toxicity Assay

S. K. Hanna, A. R. Montoro Bustos, A. W. Peterson, V. Reipa, L. D. Scanlan, S.H. Coskun, T.J. Cho, M. E. Johnson, V. A. Hackley, B. C. Nelson, M. R. Winchester, J. T. Elliott, E. J. Petersen

elijah.petersen@nist.gov

The increased use and incorporation of engineered nanoparticles (ENPs) in consumer products requires a robust assessment of their potential environmental implications. However, a lack of standardized methods for nanotoxicity testing has yielded results that are sometimes contradictory. Standard ecotoxicity assays may work appropriately for some ENPs with minimal modification but produce artifactual results for others. Therefore, understanding the robustness of assays for a range of ENPs is critical. In this study, we evaluated the performance of a standard Caenorhabditis elegans (C. elegans) developmental and reproductive toxicity assay containing an Escherichia coli (E. coli) food supply with silicon, polystyrene, and gold ENPs with different charged coatings and sizes. Of all the ENPs tested, only those with a positively charged coating caused growth inhibition. However, the positively charged ENPs were observed to heteroagglomerate with E. coli cells, suggesting that the ENPs impacted the ability of nematodes to feed, leading to a false positive toxic effect on C. elegans growth and reproduction. When the ENPs were tested in two alternate C. elegans assays that did not contain E. coli, we found greatly reduced toxicity of ENPs. This study illustrates a key unexpected artifact that may occur during nanotoxicity assays.

Materials Measurement Laboratory, National Institute of Standards and Technology

Examination of the Acute Oral Versus Sub-Acute Dietary Testing in Avian Risk Assessment Conclusions

G. Hilton[1], E. Odenkirchen[2], M. Panger[2], G. Waleko[2], A. Clippinger[1]

ginah@piscltd.org.uk

The United States Environmental Protection Agency (USEPA) requires pesticide registrants to submit toxicity data that are used to conduct ecological risk assessments. While the USEPA has required both an acute oral and sub-acute dietary tests in birds, trends over the past 20 years have indicated that the avian sub-acute dietary test generally does not drive risk management decisions. Thus, a retrospective analysis was conducted to evaluate 119 pesticides with publicly available ecological risk assessments that were registered into commerce between 1998 and 2017. Risk quotient (RQ) data from the avian acute oral and dietary tests were compared to determine if either of the tests drove the risk management decision. The RQvalues were chosen as the data point for comparison in order to assess total risk (i.e., exposure and toxicity). After comparing RQ values from avian acute oral versus sub-acute dietary tests, there were no cases found to have an avian sub-acute dietary risk quotient greater than the most conservative level of concern (LOC) for endangered species that was not also identified in the acute oral test, and 95% of the avian sub-acute dietary tests had an RQ value less than the endangered species LOC. Based on the results of the retrospective analysis, it is concluded that the avian sub-acute dietary test can be waived in most cases while still allowing for scientifically robust risk assessments and risk management decisions.

[1]PETA International Science Consortium Ltd., [2]United States Environmental Protection Agency

Rapid Evidence Mapping for Environmental Toxicology

J. Lam, B. Howard, D. Bautz, L. Anderson, A. Tandon, A. Maharana, R. Shah

ruchir.shah@sciome.com

Stakeholders in environmental health are increasingly relying on tools and practices from the disciplines of evidence synthesis and systematic review to summarize the evidence and identify scientific consensus. For example, the practice of "Evidence Mapping" is frequently used to identify key areas of study relevant to a given topic and to highlight important gaps in the literature. However, constructing detailed Evidence Maps can be a resource-intensive procedure, thereby limiting their utility for practical implementation. Here we outline a process called "Rapid Evidence Mapping" (rEM) which we define as a resource-efficient form of knowledge synthesis whereby components of the systematic review process are simplified or omitted to quickly produce a visual representation of the scientific evidence, while still utilizing rigorous, transparent, and explicit methodological approaches. Using an environmental health case study, we illustrate how rEMs can be created with hours to days of effort rather than the weeks to months typically required for a full Evidence Map or Scoping Study. We then demonstrate that the conclusions drawn using the resulting rEM are comparable to those made using a traditional Evidence Map. Our semi-automated machine learning software tools greatly reduce the resource commitment required to undertake such assessments, increasing the feasibility of their use on a widespread scale. As is often the case when comparing knowledge synthesis methodologies, there is a trade-off between rigor and speed, but we suggest here that rEMs can be extremely useful in many scenarios where preliminary assessment of the literature landscape is desired, but resources are limited.

Sciome LLC

Integration of *In Vitro* and *In Silico* Models for Predictive Toxicology in Discovery Molecule Development

J. LaRocca, K. Johnson, A. Lehman, R. Gantzer, Z. Zhang, S. Gehen

jllarocca@dow.com

The discovery and development of novel molecules is a complex, interdependent process, which requires a large investment of time and intensive animal use. Embracing high throughput predictive models for assessment of potential hazards of molecules early in discovery can help drive in decision-making, with the ultimate goal of generating products with a more favorable human health profile. To this end, we assessed the predictive utility of cytotoxicity endpoints by comparing *in vitro* IC50 values of 2D and 3D HepG2 cells to reported rat 90-day *in vivo* NOAEL and LOAELs for 22 pesticides. Using a linear mixed-effects model accounting for a grouping structure of pesticide class (fungicide, insecticide or herbicide), we demonstrated *in vitro* cytotoxicity and *in vivo* endpoints are correlated. Given that molecules can have varying absorption and bioavailability profiles, which can impact *in vivo* points of departure but are not accounted for *in vitro* systems, we integrated PBPK software to aid in placing *in vitro* cytotoxicity values into a more biologically relevant context for internal exposure. Finally, integration of transcriptomics into early-stage discovery programs can help predict points of departure for risk assessment. *In vitro* and *in vivo* points of departure for multiple fungicides were compared to *in vivo* apical endpoints, which suggest toxicogenomic bioactivity can provide an early indication of *in vivo* toxicity. These data demonstrate that harnessing *in vitro* cytotoxicity, *in silico* PBPK models, and *in vitro* and *in vivo* toxicogenomics can predict *in vivo* points of departure, which in turn aids in molecule prioritization decisions in the discovery phase.

Corteva Agriscience™, Agriculture Division of DowDuPont™

Incorporation of Absorption and Metabolism Into Liver Toxicity Prediction for Phytochemicals: A Tiered *In Silico* QSAR Approach

Y. Liu

yitong.liu@fda.hhs.gov

An increased use of herbal dietary supplements has been associated with adverse liver effects such as elevated serum enzymes and liver failure. The safety assessment for herbal dietary supplements is challenging since they often contain complex mixtures of phytochemicals, most of which have unknown pharmacokinetic and toxicological properties. Effective and rapid tools are needed to fill the data gap of evaluating large numbers of phytochemicals for potential liver toxicity. The current study demonstrates a tiered approach combining identification of phytochemicals in liver toxic botanicals, followed by *in silico* quantitative structure-activity relationship (QSAR) evaluation of these phytochemicals for absorption (e.g. permeability), metabolism (cytochromes P450) and liver toxicity (e.g. elevated transaminases). First, 255 phytochemicals from 20 botanicals associated with clinical liver injury were identified, and the phytochemical structures were subsequently used for QSAR evaluation. Among these identified phytochemicals, 193 were predicted to be absorbed and then used to generate metabolites, which were both used to predict liver toxicity. Forty-eight phytochemicals were predicted as liver toxic, either due to parent phytochemicals or metabolites. Among them, nineteen phytochemicals have previous evidence of liver toxicity (e.g. pyrrolizidine alkaloids), while the majority were newly discovered (e.g. sesquiterpenoids). These *in silico* findings help reveal new potentially toxic phytochemicals in herbal dietary supplements and prioritize future *in vitro* and *in vivo* toxicological testing.

Division of Toxicology, Office of Applied Research and Safety Assessment, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration

Microfluidic Small Airway Lung Model for Toxicity Screening of Tobacco Products

P. Makena[1], D. Gordon[2], D. Haithcock[2], B. Prabhakarpandian[2], K. Pant[2], G.L. Prasad[1]

makenap@rjrt.com

Physiologically relevant *in vitro* lung models are valuable tools to accurately assess the cytotoxicity of inhaled toxicants. Epithelial cells cultured in transwells do not accurately represent the cellular architecture, physiology, and fluidic shear present *in vivo*. Recent advances in microfluidics have led to the development of bioengineered, three-dimensional human small airway models composed of human bronchial epithelial (NHBE) cells surrounded by human lung microvascular endothelial (HMVE) cells. In this study, we evaluated the potential of a human small airway lung-on-a-chip model to assess cytotoxicity and functional endpoints following exposure to cigarette total particulate matter (TPM). The microfluidic device is comprised of a central air channel and lateral vascular channels separated by porous architecture. Using an optimized co-culture protocol, NHBE and HMVE cells were co-cultured using a combination of air and fluidic pumps. The cells were exposed apically to different concentrations of TPM for 4 hours and 1) cell viability, 2) oxidative stress, 3) cell death, and 4) epithelial permeability were assessed after 20 hours post exposure. TPM induced a dose-dependent decrease in cell viability and increased oxidative stress, cell death, and epithelial permeability. These results 1) show the potential of the human small airway *in vitro* lung model to mimic physiologically relevant conditions and 2) lay a foundation to study the effects of different tobacco products. This model enables our understanding of key molecular events that lead to early biochemical, toxicological, and/or physiological perturbations and will be useful in identifying relevant biomarkers of effect.

[1]RAI Services Company, [2]SynVivo Inc.

Tracking EPA's Progress toward Human-Relevant Chemical Testing

J. Manuppello

jmanuppello@pcrm.org

The Frank R. Lautenberg Chemical Safety for the 21st Century Act, enacted in June 2016, substantively amended the Toxic Substances Control Act for the first time in forty years. It requires EPA to reduce and replace the use of vertebrate animals in the testing of chemical substances by facilitating the use of alternative methods and strategies that provide information of equivalent or better scientific quality and relevance. Using EPA's ChemView database, we previously documented a roughly ten-fold increase in animal testing requirements for new chemicals in the first year of the amended TSCA's implementation, including in cases for which acceptable alternatives exist, such as skin sensitization and eye irritation/corrosion. The test most frequently required was the Combined Repeated Dose Toxicity with the Reproduction/Development Toxicity Screening Test, OECD Test Guideline 422, which requires approximately 580 animals.

We have updated and expanded our analysis to include chemicals reviewed since our initial report as well as information from EPA's Premanufacture Notices status table, Significant New Use Rules, and data submitted to EPA in response to its testing requirements. This has confirmed our earlier findings. For chemicals reviewed recently, the updated information is consistent with EPA relying more on pended testing requests than triggered testing requirements. (Pended testing requests describe information that EPA would require before modifying restrictions on manufacture, processing, distribution, use, and disposal of new chemicals, while triggered testing requirements are tests that EPA requires to be conducted in order to permit manufacturing new chemicals beyond specified time limits or manufacturing volume limits.) We will continue to use these resources to evaluate EPA's progress toward reducing and replacing animal use with more relevant alternatives that better protect the public from risks posed by chemicals.

The Physicians Committee for Responsible Medicine

Systematic Evaluation of Transcriptome Extrapolation Techniques

D. Mav, A. Tandon, A. Maharana, D. Phadke, B. E. Howard, R. Shah

ruchir.shah@sciome.com

Transcriptomic expression profiles can facilitate identification of chemically-induced biological responses for characterizing toxicity and disease pathways. However, the standard techniques such as Microarray and RNA-Seq remain cost-prohibitive for use in high throughput studies involving numerous experimental conditions. Recently, we reported an optimized transcriptomic subset (consisting of ~2700 genes, referred to as S1500+) that can be measured in targeted techniques such as TempO-seq. We also reported that principal component regression (PCR) extrapolation accurately models the gene to gene inter-connectedness of the transcriptome, producing reliable whole transcriptome differential expression profiles. While the first version of this approach required prior-matching of samples complicating the subsequent use of standard differential pathway detection tools; we now have updated the PCR technique by incorporating pre/post extrapolation standardization to enable baseline gene expression extrapolation. We compared its performance with a recently published deep learning (DL) technique. For DL, a multi-task multi-layer feedforward neural network consisting of one input layer, 3 hidden layers (with 3,000 hidden units each), and one output layer was customized. Series-wise RMA normalized signal for 117,559 Affymetrix microarray samples (NCBI, GEO) was used to perform 20-fold cross validation. Signal from S1500+ was used to extrapolate signal for the remaining 18,167 genes. Results indicate that PCR outperforms DL in terms of root mean square error (PCR=0.39; DL=0.51) and median absolute error (PCR=0.20; DL=0.26). Additionally, PCR is computationally less intensive, increasing its overall utility. These extrapolation efforts enable use of conventional differential expression analysis techniques requiring whole transcriptome data after only measuring a selected transcriptomic subset.

Sciome LLC

Increasing the Availability and Quality of Human Tissue in Science

J. McCarthy, K. Sullivan

jmccarthy@pcrm.org

Researchers need human tissues to conduct ethical, efficient research and to achieve breakthroughs in medicine and toxicology, ultimately saving and improving millions of lives. Human tissues are invaluable resources for medical research, diagnostic tests, biomarker discovery, drug development, product testing, chemical exposure, and much more. Fresh tissue samples are the most realistic model environment to conduct in-depth analyses of the human body.

The variation in tissue handling, processing, and characterization protocols often compromise reproducibility and effectiveness of donor tissues for various research applications. Harmonizing methods and developing a cross-industry standardization of best practices for the collection, storage, and transport of tissues will improve their integrity and maximize their potential in research. Supporting biorepositories, the development of new human tissue modelling technology, and raising awareness in the scientific and regulatory communities are key ways in which the barriers to greater uptake of human tissue models can be overcome.

On October 25, 2018 the Physicians Committee will host a Human Tissue Roundtable to draw together representatives of federal agencies, industry, academia, and patient outreach organizations. Discussions will encompass the legal, scientific, policy, and educational barriers that stifle the use of human tissues in research and toxicology. The roundtable will result in collaboration and an action plan to bring public policy into alignment with modern science and will ultimately facilitate the availability and increased use of human tissues for research, while reducing the number of animals used for experimentation.

Physicians Committee for Responsible Medicine

Unbiased High-Content Phenotyping for Personalized Chemical Exposure Risk Assessment

P. M. McLendon[1,2], D. W. Cowan[1,2], S. A. Kawamoto[2], R. H. Migliozzi[1,2], H. J. Fullenkamp[1,2], C. Kronk[1,2], C. Davidson[1,2], C. Bland[1,2], T. J. Lamkin[3], D. K. Ott[1], H. A. Pangburn[1]

pmclendon@ues.com

Background: Humans experience varying biological responses to chemical/environmental stimuli. Predicting differential responses involves understanding how an individual's genetic profile can influence diverse responses to exposure/treatment. Understanding how genetics influences response to chemical exposure at the cellular level is critical for advancing personalized medicine initiatives and next generation prognosis and/or treatment methods. This unmet need is critical in the unique environment of the warfighter, as improved predictive measures could significantly reduce the risk of chemical exposure or better inform response measures.

Methods: Genetically characterized human B-lymphoblast cell lines (LCLs) were treated for 48 hours with a panel of nine compounds representing diverse mechanisms of action over a concentration range (based upon the average LCL response). Every plate contained vehicle (0.1% DMSO) and positive controls (10% DMSO) for cell death using both Jurkat control cells and the LCL of interest. The LCLs (and Jurkat controls) were stained with viability dyes and imaged. To analyze the images and extract phenotypic information, we developed an automated high content analysis pipeline (Clarity Bioanalytics) to measure 11,000+ morphological cell features. Redundant features (i.e. those features that were similar between positive and negative controls) were eliminated, and the remaining informative features were scored, ranked, and analyzed. Clarity Bioanalytics was used to manage, analyze, and score the high content analysis data. Novel algorithms and statistical tests were used to define the phenotypic signature for each chemical toxicant and identify outlier wells (i.e. hits). Single nucleotide polymorphism (SNP) data for each LCL were downloaded from the dbSNP repository and processed for genome-wide association. Pharmacologic quantitative trait loci were mapped based on the genetic profiles of LCLs displaying "non-normal" (i.e., outliers) phenotypic signatures. The statistical relevance of various SNPs were organized by chromosome using Manhattan plots.

Results: Preliminary results from the feature evaluation and selection phase of the project indicate we can identify unbiased (sets of) features that are specific for one or more of the individual compounds. We have screened nearly 300 LCLs with the compound panel, identified phenotypic signatures that signify exposure to a specific compound, and correlated differential phenotypic responses to particular genetic polymorphisms (i.e., SNPs) using Genome Wide Association Screening (GWAS). (Continued on next page)

[1]U.S. Air Force School of Aerospace Medicine, [2]UES Inc., [3]711th Human Performance Wing/XP

Interrogation of SNPs with publicly available databases allowed us to identify genes/genetic elements and signaling pathways that may be involved in differential cellular response to toxicant exposure.

Conclusions: The results of our proof-of-concept experiments demonstrate that we can use unbiased phenotyping to identify and characterize the cellular response to chemical exposure and, using our novel analysis pipeline, correlate phenotypic changes to with genetic information. The identification of genetic correlations to differential response patterns is an important step toward personalized assessment of biological effects of chemical exposure, which will allow predictive assessment of exposure risk on the basis of genetic information.

Channel Interactions and Robust Inference for Ratiometric β-Lactamase Assay Data: A Tox21 Library Analysis

F. Melnikov[1], J. Hsieh[3], N. S. Sipes[4], P. T. Anastas[1, 2]

fjodor.melnikov@yale.edu

Ratiometric β-lactamase (BLA) reporter assays have seen increased used high throughput screening (HTS) programs for chemical assessment. While these reporters hare highly sensitive, customizable, and control for plate and well variability, the direct inference from the ratiometric BLA endpoint may be difficult to interpret. Consequently, chemical assessment and structure-activity models based on the data may be confounded by non-linear effects on different channels in the ratiometric set up. We fit and analyzed the concentration-response curves produced by the 10,000 chemicals screened in seven BLA stress-response assays as a part of the Tox21 initiative. Particular attention was given to the relationship between the three BLA assay readouts: background, target, and ratio; and uncertainty quantification. The ratio is often used solely for activity classification. However, we found that activity classifications based on a BLA ratio readout are confounded by interference patterns for as many as 85% of active chemicals in some assays. Furthermore, the potency and efficacy estimates derived from the ratio readouts may not represent the target channel effects and thus complicate chemical comparison. Therefore, we recommend a direct analysis of the target gene-expression channel to determine the target activity in a BLA assay followed by analysis of background fluorescence or viability counter screens to screen for loading or viability artifacts. The difference between viability and target gene effects is best assessed explicitly for each exposure experiment based on robust parameter uncertainties. This approach eliminates the channel interference issues and allows for straightforward chemical assessment and comparisons.

[1]School of Forestry and Environmental Studies, Yale University, [2]Department of Chemical and Environmental Engineering, Yale University, [3]Kelly Government Solutions, [4]National Toxicology Program/National Institute of Environmental Health Sciences (NIEHS)

ChemTunes ToxGPS Read-Across As A Chemoinformatics-Based Tool for Supporting Regulatory Decision Making: A Case Study of Dermal Toxicity of Alkyl PEG Ethers

A.Mostrag[1], I. Boyer[2], B. Bienfait[3], B. Heldreth[2], T. Kleinoder[3], J. Marusczyk[3], A. Tarkhov[3], O. Sacher[3], C.H. Schwab[3], V. Vitcheva[1], J.F. Rathman[3,4], C. Yang[1,3,4]

aleksandra.mostrag@mn-am.com

The applicability of *in vitro* and *in silico* methods for human safety/risk assessment is of a key interest for regulatory bodies. Read-across (RA) is one of the data gap filling techniques, involving inferring the data from a closely related analog(s) and combining various evidences (*in vivo* data, *in silico* predictions, etc.) into a single outcome. In the current study we apply the novel methodology implemented in the chemoinformatics system ChemTunes ToxGPS, to fill the data gaps for selected cosmetic ingredients from alkyl (C16-C18) PEG ethers class of compounds, using skin irritation and sensitization as target endpoints. 369 alkyl PEG ethers (including 221 ingredients not currently used but that could be used in the future) were previously evaluated by the Cosmetic Ingredient Review (CIR) Panel based on expert judgement and inference to fill the data gaps. It was concluded that all these ingredients are safe in the present practices of use and concentrations when formulated to be non-irritating. Applying ChemTunes ToxGPS Read-Across workflow enabled: (i) identification of the most mechanistically relevant and biologically meaningful analogs within alkyl PEG ethers based on molecular and physicochemical properties important for assessing dermal safety, (ii) *in silico* predictions of dermal toxicity, (iii) systematic application of the weight of evidence method to predict the final outcome, (iv) estimation of uncertainties. We demonstrate how chemoinformatics-based read-across approach can augment expert judgement and serve as a regulatory decision support tool.

[1]Altamira LLC, (V. Vitcheva's current affiliation: ECHA), [2]Cosmetic Ingredient Review, (I. Boyer's current affiliation: Keller and Heckman LLP), [3]Molecular Networks GmbH, [4]The Ohio State University

Human In Vitro Developmental Toxicity Assay Combined with a Read-Across Approach for Chemical Assessment

J.A. Palmer[1], L.A. Egnash[2], R.E. Burrier[1], F.R. Kirchner[1], E.L.R. Donley[1]

jpalmer@stemina.com

The reproductive and developmental toxicity requirements for registering chemical substances under REACH (Registration, Evaluation, Authorization, and Restriction of Chemicals) using whole-animal models could cost up to 8 billion dollars and require the use of up to 22 million animals. However, the law encourages the use of alternative methods such as *in vitro* assays and structure-activity relationship (SAR) methods for addressing data gaps and requires authorization for animal testing from the European Chemicals Agency (ECHA). The ECHA guidance documents states that alternative methods are not suitable replacements for *in vivo* testing, thus commonly used approaches include "read-across", a process in which gaps are filled using data from related compounds, and "weight of evidence" based on existing data and factors such as chemical structure and anticipated exposure levels. The utility of these approaches in developmental toxicology, when used alone, is still uncertain. Data generated from *in vitro* assays could complement these approaches.

In this study, we used a set of five structurally related 1,2,4-triazole fungicides (flusilazole, hexaconazole, propiconazole, triadimefon and myclobutanil) to demonstrate the utility of a combined testing approach. Human induced pluripotent stem cells were exposed to eight concentrations of each compound. Spent media was collected to determine changes in biomarkers of developmental toxicity (ornithine and cystine) and cell viability was measured. Historical data had shown that the assay is highly predictive of developmental toxicity potential across a diverse set of chemotypes (85% accuracy). This study demonstrates how an *in vitro* assay can be used to strengthen and enhance the read across and weight of evidences approaches used in preparation of REACH dossiers.

[1]Stemina Biomarker Discovery, Inc., [2]Current Address: Covance

Cellular Resilience and Recovery – The New Angle to Look at Toxicity

L. Smirnova, G. Harris, A. Maertens, A. Kleensang, H. Hogberg, T. Hartung

lsmirno1@jhu.edu

To date, most *in vitro* toxicity testing focuses on acute effects of compounds at high concentrations. However, measurement of cytotoxicity cannot explain different susceptibility to exposures and organ selectivity. Therefore, we hypothesised that it is rather the way how the cells can cope with the stress and respond to the next exposure makes them differently susceptible. We used a dopaminergic neurons as a model, because they cannot cope with the same stress as other types of neurons. 3D neuronal cultures overcome the limitations of monolayer cultures in studying chemical withdrawal and recovery. We used a human 3D dopaminergic LUHMES model to determine whether effects of short-term rotenone exposure (100 nM, 24 h), are permanent or reversible. A decrease in complex I activity, ATP, mitochondrial diameter and neurite outgrowth were observed acutely. After compound removal, complex I activity was still inhibited, however, ATP levels were increased, cells were electrically active and aggregates restored neurite outgrowth integrity and mitochondrial morphology. We identified significant transcriptomic changes after 24 h which were not present 7 days after wash-out. To study cellular resilience, 3D LUHMES were re-exposed to rotenone after recovery period. Pre-exposed cells maintained higher metabolic activity than controls and presented a different expression pattern of rotenone sensitive genes. In summary, our study shows that dopaminergic neurons can recover from short-term exposure to rotenone and are more resilient to a second hit, however it is not clear yet, weather this resilience is beneficial or detrimental in a long-term.

Center for Alternatives to Animal Testing, Johns Hopkins University Bloomberg School of Public Health

Phase 1 Validation of the Electrophilic Allergen Screening Assay

J. Strickland[1], J. Gordon[2], J. Hettick[3], B. Law[3], E. J. Petersen[4], N. Solomotis[5], J. Truax[1], R. Uhl[2], J. Yourick[5], D. Allen[1], N. Kleinstreuer[6]

jstrickland@ils-inc.com

Covalent binding of an electrophilic chemical to a nucleophilic binding site on skin protein is a known molecular initiating event in the skin sensitization adverse outcome pathway. The electrophilic allergen screening assay (EASA) assesses this event by measuring depletion of up to two probe chemicals displaced by a test chemical that binds to the probe(s). Probe depletion is detected using absorbance or fluorescence measurements. A test chemical is positive if the depletion of any probe is ≥30%, and negative if probe depletion is either <10% in the absorbance or <15% in the fluorescence test. Confirmatory testing is conducted when results fall between the criteria for positive and negative outcomes. NICEATM is coordinating a multi-laboratory validation study to characterize the usefulness and limitations of the EASA for skin sensitization hazard classification. Phase 1 of this study involved testing 10 coded chemicals three times in three laboratories to demonstrate reproducibility. Classifications as sensitizers or nonsensitizers were reproducible within each laboratory for 100% (10/10) of the chemicals. Among laboratories, classifications were reproducible for 90% (9/10) of the chemicals. The classifications of 70% (7/10) of the chemicals were accurate with respect to LLNA classifications in two laboratories; one laboratory's results yielded accuracy of 60% (6/10). All misclassifications were false negatives. Phase 1 data were considered adequate to progress to Phase 2. In this phase, we will develop a 96-well format to increase throughput and comprehensively assess test method accuracy. This was funded with U.S. federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.

[1]ILS, [2]CPSC, [3]CDC/NIOSH/HELD, [4]NIST, [5]FDA/CFSAN, [6]NIH/NIEHS/DNTP/NICEATM

Identifying Eye Irritants (GHS Category 2) Using Validated, Non-Animal Tests

B. Varsho, B. Yasso, M. Troese, E. Delacruz, G. DeGeorge

mbweb@mbresearch.com

OECD Test Guideline 405 prescribes a weight-of-evidence (WoE) analysis and sequential testing strategy for the classification of acute eye hazards, including, perform validated and accepted *in vitro* or *ex vivo* ocular test(s). Four tests qualify: three designed to identify severe eye irritation/corrosion (GHS Category 1) and one to identify non-irritants (GHS No Category). GHS Category 2 (eye irritant) classification is impossible using any single test. The EpiOcular Eye Irritation Test; EIT (OECD 492) classifies a substance to be No Category, or contrarily causes (uncategorizable) eye effects. Conversely, the Bovine Corneal Opacity and Permeability (BCOP) Test (OECD 437) is used for ruling in or ruling out Category 1 effects. By using a dual-assay/approach system, the combination of the EIT and BCOP test, we have determined, with a high degree of accuracy, GHS Acute Eye Hazard Category 2 chemicals that cause reversible eye irritation. When a BCOP test rules out GHS Category 1 and the EIT rules out GHS No Category, analysis of these results indicates the only other possible designation, Category 2. Per GHS, Category 2 classification defaults to Category 2A, because differentiation between Category 2A and 2B cannot be made. After testing 42 chemicals, we correctly identified 93% of the Category 2A/B chemicals as Category 2. The potential of the BCOP EIT dual-assay system, coupled with WoE evaluation, to correctly classify substances into GHS Category 1, Category 2A, and No Category is encouraging. We predict using this testing strategy would greatly reduce reliance on Draize Rabbit Eye Tests.

MB Research Laboratories

The *In Ovo* Oral Toxicity Assay: A Rat Acute Oral Systemic Toxicity Replacement

B. Varsho, C. Kalimtzis, D. Sergeyev, B. Yasso, G. DeGeorge

mbweb@mbresearch.com

Correctly identifying a chemical's acute systemic toxicity hazard level is critical for labeling the material with the proper safety precautions needed for handling and transportation. Acute toxicity categories are assigned, in part, by an oral toxicity estimate, as determined by lethality studies in rats. However, oral toxicity assays are limited. We have developed a metabolically competent assay system that models human biologic responses to exogenous chemicals such that cardiovascular, nervous, endocrine, digestive and excretory systems are represented and allowed to interact naturally *in situ*.

We administered seven reference chemicals (i.e., strychnine, caffeine, sodium nitrite, acetylsalicylic acid, ethanol, sodium chloride and sucrose) directly into the yolk and/or the albumen of non-fertilized chicken eggs on either Incubation Day (ID) 5, 6, 7 or 14, and observed the eggs for 48 hours for viability. LD50 values for each experimental condition were calculated following Log-Probit methodology. We demonstrate that reference chemicals elicit dose-responsive toxicity in eggs, and we are able to stratify lethality responses based on the relative toxicity of agents used in our experiments. Egg LD50 values were generally similar to rat LD50 values, e.g., rat oral toxicity for sodium nitrite (200 mg/kg) and caffeine (190 mg/kg) had average egg LD50 values of 136 and 237 mg/kg, respectively. We plan a robust validation to qualify IOOTA as not only an inexpensive alternative assay to mammalian hazard identification, but could serve as a stop-gap assay until sufficiently accurate *in vitro* and computer models are developed.

MB Research Laboratories

The Porcine Corneal Opacity and Reversibility Assay (PorCORA) and Assessment of the Drivers of Classification with Regards to Ocular Damage

P. Vij, D. Sergeyev, M. Carathers, E. Delacruz, B.J. Varsho, G.L.DeGeorge

mbweb@mbresearch.com

The Draize Rabbit Eye Test assesses damage to ocular structures, which are scored and weighted based on toxicological importance. The structures are: Cornea (CO), Conjunctiva (Conj), and Iris (IR). Corneal irritation is assessed by opacities on the cornea, Conj by impact on the vasculature, and IR damage by alteration of the function of the iris (constriction or dilation) and deepening of the rugae. Score weighting is according to: CO damage accounting for 80 of the 110-point Draize scale, and conjunctival effects for 20 points, with IR for 10 points. Since CO scores have the heaviest weight, and most often are the drivers of eye irritation, we developed an *ex vivo* corneal model to assess these effects. PorCORA is an *ex vivo* ocular assay which distinguishes between a substance's potential to cause severe (reversible) versus corrosive (irreversible) damage. We tested 56 chemicals ranging from corrosive (GHS category 1) to non-irritating (GHS not categorized) using Cooper Statistics. We obtained an Accuracy of 88% with a Positive and Negative Predictivity of 91% (Category 1), and 85% (not Category 1), respectively. Re-examination of our data using the *in vivo* drivers of classification concept of Barroso et. al., 2016 and its database and methodology, we found eight chemicals that had invalid tests (animals euthanized prior to day 21) or GHS classification not driven by corneal opacities. When these chemicals were curated in our dataset, the Accuracy improved to 90%, with a major change in Sensitivity, which increased from 80% to 87%.

MB Research Laboratories

Developing Highly Accurate Computational Models for Neuronal Targets

S. J. Wijeyesakere, D. Wilson, T. Auernhammer, A. Parks, and S. Marty

SWijeyesakere@dow.com

The ability to mechanistically align a xenobiotic with its target receptor(s) is a major goal of the cheminformatics and predictive toxicology fields. As an initial proof-of-concept, we sought to build such models for major neuronal targets. We mined public data sources (ToxCast, CHEMBL, BindingDB and ZINC), together with the scientific literature for compounds that interacted with the nicotinic and muscarinic acetylcholine receptors, acetylcholinesterase, GABA-A and B receptors as well as the serotonin and glycine receptors. We used structural fingerprints and two-dimensional structural scaffold-based predictions to screen compounds for their ability to interact with these receptors, followed by clustering and identification of common structural motifs. The scaffolds and fingerprints were used as a covariates in machine-learning models within KNIME workflows to predict the likelihood of novel compound interacting with the target(s). For targets with a sufficient number of active compounds, the fingerprint and scaffold-based prediction models were able to predict a positive outcome with high sensitivity (>80%). Exquisite sensitivity (>98%) and accuracy (>97%) statistics were observed for data-rich targets such as the cholinergic system. Moreover, the high sensitivity of these models correlated with high negative prediction values (>93%), underscoring the confidence with which novel compounds can be aligned with these neuronal targets. In conclusion, we demonstrate the utility and feasibility of computerized workflows to mine data and develop highly accurate prediction models to identify the sub-cellular neuronal target(s) of novel compounds.

The Dow Chemical Company, Toxicology and Environmental Research and Consulting

Multi-Behavioral Endpoint Testing of an 87-Chemical Compound Library in Freshwater Planarians

S. Zhang[1], D. Hagstrom[1], E. S. Collins[1,2]

ecollin3@swarthmore.edu

We have developed the asexual freshwater planarian Dugesia japonica as an alternative invertebrate model for developmental neurotoxicology, using fast screening of multiple behavioral endpoints, developmental toxicity, and mortality. Using an 87-compound library provided by the National Toxicology Program, consisting of known and suspected neurotoxicants, including drugs, flame retardants, industrial chemicals, polycyclic aromatic hydrocarbons (PAHs), pesticides, and presumptive negative controls, we evaluate the benefits and limitations of the system. We show that, in the context of this library, planarians are the most sensitive to pesticides with 16/16 compounds causing toxicity and the least sensitive to PAHs, with only 5/17 causing toxicity. Furthermore, while none of the presumptive negative controls were bioactive in adult planarians, 2/5, acetaminophen and acetylsalicylic acid, were bioactive in regenerating worms. Notably, these compounds were previously reported as developmentally toxic in mammalian studies. Through parallel screening of adults and developing animals, planarians are thus a useful model to detect such developmental-specific effects, which was observed for 13 chemicals in this library.

[1]University of California, [2]Swarthmore College

ASCCT Awards

One of the main aims of the ASCCT is to support and engage young scientists working in the in vitro and computational toxicology fields. To do this we provide financial awards and travel bursaries to our annual meeting as well as other topical meetings.

Edward Carney Predictive Toxicology Award

In 2015, in memoriam of Dr. Edward Carney, the Society established the Edward Carney Predictive Toxicity Award. Dr. Carney was an active and dedicated member of the ASCCT, and a partner, mentor, and friend who inspired many in our fields. The award is $500, and will be awarded to a winning first author presenting at each annual ASCCT meeting, to assist with travel and/or research expenses.

Previous Award Winners

2015: Dr. Nicole Kleinstreuer: Identifying reference chemicals for androgen receptor activity

2016: Emma Bowers: Modeling a complex in vivo response in vitro: Exploring heterogeneity and mechanisms associated with ozone adaptation

2017: Ellen Garcia: Characterization of two lung cell lines for use in cell division focused, single-cell toxicity assays

Tox 21 Student Award

After winning the William and Eleanor Cave Award for his career achievements in in vitro toxicology, Dr. Ray Tice generously established the Tox21 Student Award. Under this award, $500 will be given at each annual meeting for the next five years for the best student presentation.

Previous Award Winners

2016: Ellen Garcia: Single-cell analysis reveals that silver nanoparticle exposure leads to multi-nucleation through defective cell division

2017: Wenyi Wang: Mechanistic evaluation of chemicals that induce oral acute toxicity by mitochondrial membrane disruption: Big data profiling and analysis

2018 ASCCT Notes

2018 ASCCT Notes

IIVS
Institute for In Vitro Sciences

RAI
Services
Company

SOT
Society of
Toxicology
Creating a Safer and Healthier World by Advancing
the Science and Increasing the Impact of Toxicology

Physicians
Committee
for Responsible Medicine
PhysiciansCommittee.org

insphero
Biomimetic Microtissue Technology

ILS
Advancing Science, Improving Lives

ALTERNATIVES
RESEARCH & DEVELOPMENT
FOUNDATION