6th Annual Meeting
of the
American Society for Cellular and Computational Toxicology

September 21-22, 2017

Institute for In Vitro Sciences
Gaithersburg, Maryland

President's Welcome

Welcome to the 6th Annual Meeting of the American Society for Cellular and Computational Toxicology. It's hard to believe a year has passed since we last met, but there certainly has been a tremendous amount of activity in the area of alternative methods over the past 12 months. Our Annual Meeting returns this month to the metropolitan Washington, D.C. area as we convene at the Institute for In Vitro Sciences, one of our founding sponsors, in Gaithersburg, MD.

The organizing committee has put together an excellent program with presentations that are arranged into two themes: New Horizons in Acute Toxicology: Research and Policy Advances; and Meeting the Challenges of the Lautenberg Chemical Safety Act. The program kicks off with plenary presentations from experts in U.S. regulatory toxicology summarizing two major initiatives within the Federal government to replace traditional animal tests. This will be followed by platform presentations from ASCCT members highlighting their work. We'll close the meeting on Day 2 with a Panel discussion focused on strategic implementation of *in vitro* and *in silico* methods for industrial chemical testing under the Lautenberg Act.

Please make sure to take advantage of the poster discussion session on Thursday evening that is always a great time to network with your colleagues and discuss cutting-edge science. We'll again recognize two outstanding young scientists with the Edward Carney Predictive Toxicology Award, an annual award started in 2015 to recognize an outstanding poster presentation in memory of our friend and colleague; and the Tox21 Student Award, established in 2016 by our good friend and longtime ASCCT member, Ray Tice. Speaking of students, after last year's successful event, we'll again set aside a networking luncheon to allow young scientists to interact with many of our more experienced members.

There's a long list of people that have contributed to making this meeting happen, but none more important that than my fellow ASCCT members that served on this year's organizing committee. Please join me in thanking the organizing committee and Board of Directors, and thank you for coming to share your work and get involved in the Society. And, don't forget to thank Kristie for her continued organization and oversight of the outstanding webinars that we've all enjoyed over the past year. Finally, I want to thank each of you for becoming ASCCT members and for your continued contributions to our Society, which now stands at well over 250 members!

The ASCCT continues to be a platform to exchange ideas among regulatory and research scientists from both the computational and cellular sides of toxicology. Let's make sure the next year continues 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 strengthens cooperation between cosmetic, pharmaceutical, and chemical industry scientists and professionals.

Have a great meeting!
Dave

PS: Make sure and check out the new and improved ASCCT website at www.ascctox.org!

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2017 ASCCT Agenda

Thursday, September 21

9:00-9:15	President's Welcome | David Allen, Integrated Laboratory Systems, Inc.
9:15-11:30	**Opening Session: New Horizons in Acute Toxicology: Research and Policy Advances** **Chair: Shaun McCullough**
9:15-10:15	EPA-OPP's Initiative to Modernize the Acute "6-Pack" | *Anna Lowit, US Environmental Protection Agency, Office of Pesticide Programs*
	Progress Report on Efforts to Replace Acute Systemic Toxicity Tests with Mechanistic Alternative Approaches | *Dan Wilson, Dow Chemical Company*
10:15-10:30	Discussion
10:30-12:00	**Oral Presentations: Acute Toxicity Testing (15 minutes each)**
	Moving Beyond the 6-Pack for Evaluating Acute Toxicity of Agrochemicals | *Sean Gehen, Dow AgroSciences LLC*
	Characterizing Variability Across Rat Oral Acute Toxicity Studies: Implications for Alternative Model Evaluation | *Agnes Karmaus, Integrated Laboratory Systems, Inc.*
	Mechanistic Evaluation of Chemicals that Induce Oral Acute Toxicity by Mitochondrial Membrane Disruption: Big Data Profiling and Analysis | *Wenyi Wang, Rutgers University*
	Implementing Alternative Approaches for Inhalation Toxicity Testing: Recent Success and Remaining Hurdles | *Amy Clippinger, PETA International Science Consortium, Ltd.*
12:00-2:00	**Lunch, Poster Viewing & Mentoring Activity** Posters attended 1:00-2:00 PM
2:00-2:30	O Canada: A Strategic Vision Toward Replacement in the True North | *Charu Chandrasekera, Canadian Centre for Alternatives to Animal Methods*

2:30-3:30	**Oral Presentations: Free Communications (20 minutes each)** **Chair: Ellen Berg** A Tiered Approach to *In Vitro*-based Safety Assessments: A Case Study on Using Fit-for-purpose *In Vitro* assays, IVIVE and Exposure Models to Evaluate Zones of Safe Exposure with Xenoestrogens | *Rebecca Clewell, Scitovation, Inc.* A New Tool for Aligning Assay Endpoints to Adverse Outcome Pathways | *Shannon Bell, Integrated Laboratory Systems, Inc.* Automated Read-Across for REACH | *Thomas Luechtefeld, Center for Alternatives to Animal Testing, Johns Hopkins University*
3:30-4:00	Break
4:00-5:00	**Oral Presentations: Free Communications (20 minutes each)** Developmental Toxicity Potency of Valproate Analogues in a Human Pluripotent Stem Cell-based Assay | *Nicole Kleinstreuer, NTP Interagency Center for the Evaluation of Alternative Toxicological Methods* Toward Achieving Harmonization in a Nano-cytotoxicity Assay Measurement Through an Interlaboratory Study | *John Elliott, National Institute of Standards and Technology* A High-Throughput Microfluidic 3D Human Liver Tissue Model for Hepatotoxicity Prediction | *Anthony Saleh, Mimetas US Inc.*
5:00-5:30	**ASCCT Business Meeting**
5:30-7:00	**Evening Reception** **Awards Ceremony** - Edward Carney Predictive Toxicology Award - Tox21 Student Award **Poster Viewing & Networking**

2017 ASCCT Agenda

Friday, September 22

Meeting the Challenges of the Lautenberg Chemical Safety Act

9:00-9:30 Progress Report on Development of a Strategic Plan to Reduce, Refine or Replace Use of Vertebrate Animal Testing Under the Lautenberg Act | *Louis (Gino) Scarano, US Environmental Protection Agency*

9:30-11:00 **Oral Presentations (18 minutes each)**
Chair: Gertrude-Emilia Costin

Implications of the Recent 2016 Amendment of the Toxic Substances Control Act (TSCA) on the Development and Implementation of Non-Animal Testing Methods | *Catherine Willett, Humane Society of the United States*

Proposal of a Comprehensive Mode-of-action Model to Aid in Occupational Health and Safety Assessments of Airway Irritants | *Evan Frank, University of Cincinnati College of Medicine*

Application of PBPK Models to Support the New Safety Assessment Paradigm – Providing a Quantitative Bridge from *In Vitro* Concentration to Human Exposure in a Tiered Manner | *Miyoung Yoon, Scitovation LLC*

A High-Throughput Microfluidic Platform for 3D Renal Proximal Tubule Toxicity Modeling | *John Lowman, Mimetas US Inc.*

Vision for U.S. Strategic Plan to Implement Alternative Toxicology Testing | *Esther Haugabrooks, Physicians Committee for Responsible Medicine*

11:00-12:30 **Closing Panelist and Audience Discussion**

- Advice from EPA on its strategic plan for implementation of alternative methods
- Necessary stakeholder contributions for successful progress
- Scientific and non-scientific challenges and solutions

Moderators:
Rodger Curren and Kristie Sullivan

Panelists:
Rick Becker, *American Chemistry Council*
Nicole Kleinstreuer, *NICEATM*
Jennifer McPartland, *US Environmental Defense Fund*
Gino Scarano, US *Environmental Protection Agency*
Catherine Willett, *Humane Society of the United States*

12:30-1:30 **Optional tour of Institute for In Vitro Sciences Laboratories**

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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

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Plenary Abstracts

Alternatives to Acute Systemic Toxicity: EPA's Office of Pesticide Programs Initiative to Modernize the Acute 6-Pack

Anna B. Lowit, Senior Science Advisor
US Environmental Protection Agency, Office of Pesticide Programs, Washington, DC

In a March 2016 open letter to stakeholders, former EPA's OPP Office Director, Jack Housenger, detailed OPP's efforts to modernize the acute toxicity six-pack studies and reduce animal testing. This letter committed OPP to explore opportunities to reduce barriers to alternative methods to animal testing and facilitate the use of OECD *in vitro* methods, including consideration of GHS. In collaboration with NTP's Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), industry and non-governmental organizations, the EPA is making significant progress towards the adoption of integrated approaches to testing and assessment (IATA) and the reduction of the use of animals in acute toxicity testing. As part of OPP's commitment to reduce animal use, in 2016, EPA completed a retrospective analysis of acute dermal toxicity studies and published a guidance document including a policy statement to waive all acute lethality dermal studies for formulated pesticide products. The Agency is currently conducting a pilot utilizing the GHS dose additive mixtures equation to reduce animal testing for product formulations. The goal of the pilot is to evaluate the utility and acceptability of the GHS dose additive mixtures equation as an alternative to animal oral and inhalation toxicity studies for pesticide product formulations. EPA is interested in expanding its use of OECD's test guidelines for *in vitro* (non-animal) studies for eye irritation, skin irritation, and skin sensitization. However, these OECD guidelines were developed and validated according to the GHS categories. Thus, EPA must develop cross-walks between the US and GHS category systems, which has been a resource intensive and time-consuming process. EPA continues to explore ways to remove the barriers to facilitating international harmonization, adoption of alternative test methods, utilizing faster, more effective, and better performing test methods while reducing the reliance on animal testing for pesticides. OPP is also committed to maintaining continuity with current programmatic efforts tied to its acute toxicity regulatory framework, such as the Worker Protection Standard (WPS), and School Integrated Pest Management (IPM) programs, and is pursuing a systematic process with stakeholder input that would balance these efforts while creating flexibility in OPP's acute toxicity classification system.

Efforts to Replace Acute Systemic Toxicity Tests with Mechanistic Alternative Approaches

Dan Wilson, Sanjeeva Wijeyesakere, Amanda Parks, Tyler Auernhammer, Sue Marty
The Dow Chemical Company

Acute mammalian toxicity is not adequately predicted by current computational or *in vitro* models. Our primary focus is on understanding the explicit molecular initiating events (MIEs) and adverse outcome pathways (AOPs) that drive acute systemic toxicity. We derived five broad mechanistic classes: (1) facile chemical reactivity, (2) surfactants, (3) chelation, (4) non-specific hydrophobic interactions, and (5) specific interactions with proteins targets. We derived automated KNIME workflows for data acquisition and structural scaffolding to identify respective compounds, and are developing prediction models using molecular docking or simple regression plots within regional compound classes. For example, we implemented our workflow to identify chemically reactive compounds in acute toxicity databases and demonstrate a higher incidence in more severe GHS classes for inhalation compared to oral exposure routes, which we had hypothesized. As a proof-of-concept for the fifth mechanistic class that contains many protein targets, we are focused first on cholinergic compounds. Using scaffolding alone, we demonstrate the ability to determine if an unknown compound would or wouldn't act as a nicotinic, muscarinic, or acetyl-cholinesterase inhibitor. Since acute mammalian toxicity data for any given MIE and route are limited, we are developing integrated workflows that associate computational mechanistic screening with targeted *in vitro* assays.

Progress Report on the Development of a Strategic Plan to Reduce, Refine or Replace the Use of Vertebrate Animal Testing Under the Lautenberg Act

Louis Scarano
U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention (OCSPP), Office of Pollution Prevention and Toxics (OPPT)

On June 22, 2016, the Frank R. Lautenberg Chemical Safety for the 21st Century Act, which amended the 1976 Toxic Substances Control Act (TSCA), the nation's primary chemicals management law, was signed into law. Along with new requirements and deadlines for actions related to the regulation of new and existing chemicals in the U.S., the new law includes changes to TSCA § 4 (Testing of Chemical Substances and Mixtures). Specifically, a new section (4 (h)) has been added entitled Reduction of Testing on Vertebrates. This new section states, in part, that EPA must "...develop a strategic plan to promote the development and implementation of alternative test methods and strategies to reduce, refine, or replace vertebrate animal testing and provide information of equivalent or better scientific quality and relevance for assessing risks of injury to health or the environment...". The Strategic Plan must be completed by June 22, 2018. The Agency plans to have a stakeholder workshop/ public meeting on November 2-3, 2017 at the National Institutes of Health (NIH) in Bethesda, MD. This talk will provide an update on the progress made in developing information that will be disseminated prior to the November workshop, including a draft goal statement and objectives of the Strategic Plan.

O Canada: A Strategic Vision Toward Replacement in the True North

Charu Chandrasekera, Ph.D.
Canadian Centre for Alternatives to Animal Methods, University of Windsor

From the Americas to the Far East, many countries boast alternatives centres, but Canada has lagged behind, until now. We recently established the Canadian Centre for Alternatives to Animal Methods (CCAAM), and its subsidiary, the Canadian Centre for the Validation of Alternative Methods (CCVAM) at the University of Windsor in Windsor, Ontario, Canada. The vision of CCAAM is to promote the replacement of animals in Canadian biomedical research, education, and regulatory testing through 21st century science, innovation, and ethics. CCAAM will serve as a leader and nexus for alternatives to animal testing in Canada by collaborating with academic, industry, non-profit, and government sectors as well as ethicists, policy makers, and the public to develop, validate, and promote ways to reduce and replace animal use through extensive Research, Academic, and Regulatory initiatives. Through our multifaceted interdisciplinary collaborative partnerships, we will enhance the Canadian replacement landscape while contributing to global replacement efforts in a uniquely Canadian way.

ASCCT 2017

Oral Abstracts

Moving Beyond the 6-Pack for Evaluating Acute Toxicity of Agrochemicals

Sean C. Gehen[1]*; Marco Corvaro*[1]

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A six pack of acute toxicity studies is required for classification and labeling of crop protection active ingredients and formulated products by most global regulatory agencies. Historically these have been animal-intensive studies; however, recent advancements in alternative toxicology methods provide the opportunity to greatly reduce or eliminate altogether animal use for acute toxicity testing in the crop protection sector. To accomplish this, alternative test methods need to be appropriately evaluated to ensure their suitability to regulatory decision making and subsequently adopted as acceptable replacements to the current animal-based test methods. This requires engagement and cooperation with industry, regulatory agencies and other interested stake-holders. This talk will highlight recent efforts within Dow AgroSciences to evaluate the performance of alternative testing approaches as well as recent regulatory policy decisions that have served to promote greater adoption of alternative approaches and reduce animal testing. The talk will focus specifically on development of integrated *in vitro* testing approaches for eye irritation and skin sensitization, calculation-based approaches for estimating systemic toxicity (oral, inhalation routes) of mixtures, as well as recent guidance supporting removal of the acute dermal toxicity study as a default requirement for formulated products. Additionally, the talk will identify opportunities for future research and policy guidance approaches, as well as opportunities for global harmonization of approaches.

[1]*Dow AgroSciences, LLC*

Characterizing Variability Across Rat Oral Acute Toxicity Studies: Implications for Alternative Model Evaluation

Agnes L. Karmaus, Ph.D.[1], David G. Allen, Ph.D.[1], Nicole C. Kleinstreuer, Ph.D.[2], Warren M. Casey, Ph.D.[2]

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Alternative models developed for estimating acute systemic toxicity are often evaluated using *in vivo* LD50 values as reference data. However, *in vivo* acute systemic toxicity studies conducted according to accepted test guidelines can produce results that vary significantly. This variability can make a fair assessment of alternative models difficult or impossible. To characterize the variability of *in vivo* acute systemic toxicity data, we examined LD50 values from rat oral acute toxicity studies. Data were obtained from multiple databases including the NLM's Hazardous Substances Data Bank and ChemIDplus, the OECD's eChemPortal, and the JRC's AcutoxBase, resulting in a dataset comprising a total of 28,320 oral LD50 values representing 11,686 unique chemicals. A subset of 1592 chemicals that were evaluated in at least three independent rat oral acute toxicity studies were used for assessing variability, of which 22% (343/1542) had at least one study generating an outlier LD50 value (i.e., falling outside 1.5 times the interquartile range of the distribution of LD50 values per chemical). Furthermore, 34 chemicals had LD50 values ranging across at least three orders of magnitude. Such variability resulted in 83 chemicals being classified into at least three GHS oral acute toxicity labeling categories and 53 chemicals classified into at least three different EPA categories. These findings underscore the importance of considering an appropriate margin of uncertainty when using *in vivo* oral acute toxicity data to assess the performance of alternative methods. U.S. Federal funds from NIEHS/NIH/HHS contract HHSN273201500010C supported this study.

[1]ILS, [2]NIH/NIEHS/DNTP/NICEATM

Mechanistic Evaluation of Chemicals that Induce Oral Acute Toxicity by Mitochondrial Membrane Disruption: Big Data Profiling and Analysis

Wenyi Wang[1], Dan Russo[1], Ruili Huang[2], Menghang Xia[2], Hao Zhu[1,3]

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Assessment of chemical toxicity using *in vitro* assays or *in silico* models is of great interest asalternatives to traditional animal models. However, due to the complexity of animal toxicity, neither approach has acceptable predictive accuracy to evaluate new compounds for most animal toxicity endpoints. We developed a mechanism-driven *in silico* approach for evaluating acute animal toxicity, by using quantitative High-Throughput Screening (qHTS) data from the Tox21 mitochondrial membrane potential disruption (MMP) assay, and combining all publically available toxicity assay data. Firstly, we use the in-house rat oral toxicity database of 4,647 compounds and the MMP qHTS dataset of 7,320 compounds to automatically search against PubChem, the largest public chemical data sharing portal, for all *in vitro* assay data to establish bioprofiles for compounds found in both databases. After optimizing the bioprofiles, compounds were clustered into various subsets based on their structural information. Using a bioprofile mining approach, we evaluated animal toxicants with specific chemical features within each cluster of compounds. In this procedure some structural and biological features were identified to serve as potential indicators of the toxicity pathways related to MMP, which can result in acute toxicity. This strategy can not only build predictive models for animal toxicity, but also indicate potential toxicity mechanisms by linking the chemical and biological profiles to both cellular responses and animal toxicity phenomena.

[1]The Rutgers Center for Computational and Integrative Biology, [2]National Center for Advancing Translational Sciences, National Institutes of Health, [3]Department of Chemistry, Rutgers University

Implementing Alternative Approaches for Inhalation Toxicity Testing: Recent Success and Remaining Hurdles

Amy Clippinger, Ph.D.[1], *David G. Allen, Ph.D.*[2]

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Inhalation toxicity testing provides the basis for hazard labeling and risk management of chemicals with potential exposure to the respiratory tract. The traditional *in vivo* tests commonly included in regulatory test guidelines generate an LC50, which is the concentration that causes lethality in 50% of the animals tested. Work is underway to develop mechanistically based, non-animal approaches for inhalation toxicity testing that will provide more human-relevant data and a better understanding of the mechanism of toxicity. This presentation will explore current inhalation toxicity testing requirements and describe ongoing efforts to achieve global regulatory acceptance for non-animal approaches to inhalation toxicity testing. Integrated approaches that combine the use of existing data with *in vitro* and/or computational approaches will be discussed, along with a strategy for identifying and overcoming obstacles to replacing animal testing. Specifically, the presentation will highlight available reference data sources, use of a mathematical approach to classify mixtures based on ingredient toxicity, use of three-dimensional human reconstructed lung tissue models in air-liquid interface exposure systems, and applying adverse outcome pathways as a framework for toxicity assessment. Information gaps that have hindered the global implementation of such approaches will also be highlighted. Portions of this project were funded with U.S. Federal funds from NIEHS/NIH/HHS under Contract HHSN273201500010C.

[1]*PETA International Science Consortium, Ltd,* [2]*ILS*

A Tiered Approach to *In Vitro*-Based Safety Assessments: A Case Study on Using Fit-for-Purpose *In Vitro* Assays, IVIVE and Exposure Models to Evaluate Zones of Safe Exposure with Xenoestrogens

Rebecca A. Clewell[1], *Kamel Mansouri*[1], *Daniel Doheny1*, *Michelle M. Miller*[1], *Miyoung Yoon*[1], *Patrick D. McMullen*[1]

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Catalyzed by the NAS reports Toxicity Testing in the 21st Century (2007) and Exposure Science in the 21st Century (2011), the regulatory and scientific communities are developing safety assessment strategies that employ computational models and *in vitro* human cell based assays. Based on the last several years of work with ACC LRI and other industry partners, we have developed a tiered testing strategy that fundamentally incorporates *in silico* and *in vitro* technologies into the risk assessment paradigm. This tiered approach moves from lower tiers focused on rapid decision making and prioritization (Tier 0 – 1) to higher tiers with increased biological complexity focused on improving accuracy and providing the necessary dose-response information for making chemical safety decisions. In our testing paradigm, Tier 2 uses fit-for-purpose *in vitro* assays designed to recapitulate *in vivo* human response to conduct in-depth dose-response evaluation of key toxicity pathways. Coupled with human relevant metabolism and quantitative IVIVE (Q-IVIVE), which accounts for metabolite generation and bioactivation, these Tier 2 dose-response studies are expected to support prediction of regions of safety – or exposure concentrations at which no increased risk is expected in humans. To support such decisions, Tier 2 assays should recapitulate not only a particular *in vivo* phenotype, but also the human relevant concentration-response for chemical effects. This presentation describes the process of developing and validating such fit-for-purpose *in vitro* assays. Finally, we demonstrate application of the tiered approach using a case study with estrogenic compounds, using computational approaches for prioritization and testing.

[1]*ScitoVation, LLC*

A New Tool for Aligning Assay Endpoints to Adverse Outcome Pathways

Shannon Bell, Ph.D.[1], Lyle Burgoon, Ph.D.[2], Jason Phillips, B.S.[3], Patricia Ceger, M.S.[1], David Allen, Ph.D.[1], Warren Casey, Ph.D.[4], Nicole Kleinstreuer, Ph.D.[4]

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A critical challenge to the implementation of non-animal approaches in chemical safety testing is linking endpoints measured in these approaches to adverse physiological responses *in vivo*. The adverse outcome pathway (AOP) framework allows these molecular, cellular, and tissue-level endpoints to be placed in a biologically relevant context. The National Toxicology Program's Integrated Chemical Environment (ICE) web resource houses curated data from *in vivo, in vitro*, and *in silico* endpoints. A new feature of ICE maps assay endpoints to key events within AOPs. This feature can be used to identify data gaps and build confidence in mechanistic plausibility and relevance. This new ICE feature enables use of ICE with AOPXplorer, a Cytoscape plugin that allows visualization of data in AOP networks to build confidence in the mechanistic plausibility and relevance of a proposed defined approach. Our presentation will use the skin sensitization AOP and putative AOPs for androgen and estrogen receptor pathways to demonstrate the utility of this feature. This was funded with U.S. federal funds from the NIEHS/NIH/HHS under Contract HHSN273201500010C.

[1]ILS, [2]U.S. Army Engineer Research and Development Center, [3]Sciome LLC, [4]NIH/NIEHS/DNTP/NICEATM

Automated Read-Across for REACH

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A new predictive toxicology tool, REACHAcross, a collaboration between UL and researchers from Johns Hopkins University, will contribute strongly to reduced animal use. REACHAcross was initially created using the publically available toxicology data from nearly 10,000 chemicals registered under REACH and has since been expanded to include toxicology data from other public databases. This highly dense chemical map allows finding similar molecules for most structures. The biological data available in these datasets combined with *in vivo* endpoints from REACH represent an enormous modeling potential: both chemical and biological similarity can be used to interpolate properties of structures. This approach is based strictly on the local validity of similar chemicals but offers a coverage of large parts of the chemical universe similar to a QSAR. It expresses the certainty of a prediction based on the local data around the substance of interest. This web-based tool could help evaluate the risks posed by new chemicals even before synthesizing them, identifying safer alternatives for greener chemical development and reducing the need for animal testing.

[1]*Center for Alternatives to Animal Testing at Johns Hopkins University*

Developmental Toxicity Potency of Valproate Analogues in a Human Pluripotent Stem Cell-Based Assay

Nicole Kleinstreuer[1], Jessica Palmer[2], Alan Smith[2], Robert Burrier[2], Elizabeth Donley[2], Fred Kirchner[2], Dinant Kroese[3], Regina Stöber[4]

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The development and use of alternative models for safety screening in place of animal models has been at the forefront of the toxicology field for over a decade; however, application of these assays in a regulatory setting is still poorly understood. The EU-ToxRisk project has developed several case studies to address this issue. One of these case studies investigates the teratogenic potency of several valproate (VPA) analogues. The devTOX quickPredict platform is an *in vitro* human pluripotent stem (hPS) cell-based assay that predicts the developmental toxicity potential of chemicals based on changes in hPS cell metabolism. The assay has been used by multiple industries and, of note, by the United States Environmental Protection Agency (EPA) and National Toxicology Program (NTP) in support of Tox21. In this study, we tested ten VPA analogues included in the EU-ToxRisk case study with the devTOX quickPredict platform and ranked their developmental toxicity potential. Historical data has shown that the assay is highly concordant (~85%) with human and *in vivo* developmental toxicity outcomes across a diverse set of chemotypes.

[1]National Toxicology Program Interagency Center for Evaluation of Alternative Toxicological Methods, [2]Stemina Biomarker Discovery, [3]The Netherlands Organization for Applied Scientific Research (TNO), [4]IfADo - Leibniz Research Centre for Working Environment and Human Factors

Toward Achieving Harmonization in a Nano-Cytotoxicity Assay Measurement Through an Interlaboratory Study

John T. Elliott[1], Matthias Rösslein[2], Nam Woong Song[3], Blaza Toman[4], Agnieszka Kinsner-Ovaskainen[5], Rawiwan Maniratanachote[6], Marc L. Salit[1,7], Elijah J. Petersen[1], Fatima Sequeira[1], Erica L. Romsos[8], Soo Jin Kim[3], Nadia R. Von Moos[9], François Rossi[5], Cordula Hirsch[2], Harald F. Krug[10], Wongsakorn[6], Peter Wick[2]

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Design and development of reliable cell-based nanotoxicology assays are important for evaluation of potentially hazardous engineered nanomaterials. Challenges to producing a reliable assay protocol include working with nanoparticle dispersions and living cell lines, and the potential for nano-related interference effects. Here we demonstrate the use of a 96-well plate design with several measurement controls and an interlaboratory comparison study involving five laboratories to characterize the robustness of a nano-cytotoxicity MTS cell viability assay. The consensus EC50 values were 22.1 mg/L (95 % confidence intervals 16.9 mg/L to 27.2 mg/L) and 52.6 mg/L (44.1 mg/L to 62.6 mg/L) for the A549 cell line from ATCC for positively charged polystyrene nanoparticles for the serum free and serum conditions, respectively, and were 49.7 μmol/L (47.5 μmol/L to 51.5 μmol/L) and 77.0 μmol/L (54.3 μmol/L to 99.4 μmol/L) for positive chemical control cadmium sulfate for the serum free and serum conditions, respectively. Results from the measurement controls can be used to evaluate the sources of variability and their relative magnitudes within and between laboratories. This information revealed steps of the protocol that may need to be modified to improve the overall robustness and precision. The results suggest that protocol details such as cell line ID, media exchange, cell handling, and nanoparticle dispersion are critical to ensure protocol robustness and comparability of nano-cytotoxicity assay results. The combination of system control measurements and interlaboratory comparison data yielded insights that would not have been available by either approach by itself.

[1]Biosystems and Biomaterials Division, Material Measurement Laboratory, National Institute of Standards and Technology, [2]EMPA, Swiss Federal Laboratories for Material Testing and Research, Particles-Biology Interactions Laboratory, [3]Center for Nanosafety Metrology, Korea Research Institute of Standards and Science, [4]Statistical Engineering Division, Information Technology Laboratory, National Institute of Standards and Technology, [5]European Commission, Joint Research Centre, Institute for Health and Consumer Protection, Nanbiosciences Unit, [6]National Nanotechnology Center, National Science and Technology Development Agency, [7]Department of Bioengineering, Stanford University, [8]Biomolecular Measurement Division Material Measurement Laboratory, National Institute of Standards and Technology, [9]Powder Technology Laboratory, Ecole Polytechnique Fédérale de Lusanne, [10]EMPA, Swiss Federal Laboratories for Material Testing and Research, International Research Cooperations Manager

A High-Throughput Microfluidic 3D Human Liver Tissue Model for Hepatotoxicity Prediction

Anthony D. Saleh, Ph.D.[1], *Karlijn Wilschut, Ph.D.*[1], *Henriette Lanz, Ph.D.*[1], *Paul Vulto, Ph.D.*[1], *John Lowman, B.S.*[1], *Remko van Vught, Ph.D*[1]

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Accurate predication of hepatotoxicity is a major problem in pharmacology. Animal hepatotoxicity testing is expensive and overall has unreliable concordance with human hepatotoxicity, while standard in vitro systems have, at best, marginally improved productivity. Here, we describe a 4-cell, layered model of the liver sinusoid, including hepatocytes, endothelial, Kupffer, and stellate cells in MIMETAS'high-throughput microfluidic OrganoPlate® platform. In OrganoPlates™, extracellular matrix (ECM) gels can be freely patterned in microchambers using capillary pressure barriers, providing boundary-free channels for passive medium perfusion. The microfluidic channel dimensions not only allow solid tissue and barrier formation, but also the growth of tubular epithelial vessel structures. Utilizing the OrganoPlate™ technology, we can mimic the structure of the liver sinusoid by culturing hepatocyte and stellate cells in an extracellular matrix protein gel, fed by nutrient perfusion from an adjacent endothelial and Kupffer cell-lined blood vessel mimic. The resulting platform contains 96 individual, 3D microfluidic co-cultures of human primary or iPS hepatocytes, with a microenvironment featuring three non-parenchymal human liver cell types, providing an unparalleled combination of *in vitro* physiological relevance and throughput. We report long-term maintenance of metabolic activity (by measurement of CYP3A4) and liver specific function (by albumin production), along with multi-parameter high-content imaging-based readouts of toxicity, including mitochondrial function and steatosis. Our 3D human liver model OrganoPlates™ will be a powerful screening platform for the assessment of pharmaceutical hepatotoxicity.

[1]*Mimetas US Inc.*

Implications of the Recent 2016 Amendment of the Toxic Substances Control Act (TSCA) on the Development and Implementation of Non-Animal Testing Methods

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The Frank R. Lautenberg Chemical Safety for the 21st Century Act (LCSA) increases the authority of the Environmental Protection Agency (EPA) to obtain information on new and existing industrial chemicals and could result in vast increases in animal testing. The amendment also requires both EPA and any person developing information under the LSCA to reduce and replace vertebrate testing and requires EPA to promote the development and timely incorporation of non-animal methods. Existing chemicals are subject to prioritization and high priority chemicals must undergo risk evaluation, both under tight deadlines. EPA is currently formulating the prioritization process, and will be requesting stakeholder input. This talk will describe the application of best practices drawn from several existing prioritization schemes that could be implemented by the US EPA to satisfy both the letter and the intent of the new legislation, including the mandate to minimize animal testing.

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Proposal of a Comprehensive Mode-of-Action Model to Aid in Occupational Health and Safety Assessments of Airway Irritants

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Airway disease related to exposure to chemical irritants is the most common manifestation of occupational illness and respiratory tract irritation is frequently used as a critical effect in setting airborne occupational exposure limit values. This process currently involves testing in animal models and human subjects, both of which are expensive and time-consuming methods. The biological basis of irritant-induced occupational airway conditions, which include chronic cough, non-allergic rhinitis, and irritant asthma, is complex and a comprehensive understanding of mechanisms enables more efficient and accurate hazard assessment. This review proposes a mode-of-action pathway model that integrates diverse irritant classes into a mechanistic framework to support development of low-cost alternatives to animal and human testing. We propose a pathway model where signaling cross-talk between chemosensation in nociceptive nerves and inflammation in airway epithelium mediates long-lived adverse outcomes, therefore toxic initiating events which cause signaling input into these pathways may cause or influence the potential for airway disease. Toxic initiating events include engagement of transient receptor potential (TRP) channels, oxidation of organelles, and initiation of inflammatory signaling in epithelial and immune cells. The overall hypothesis of this project is that assessment of a toxicant's activity through these mechanisms using low-cost *in vitro* tests can help predict *in vivo* airway irritancy and risk of adverse outcomes in humans. A low-cost, easily reproducible system validating this hypothesis will be of high value in screening, prioritization, and estimation of relative potency among chemical irritants and data-poor occupational toxicants.

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Application of PBPK Models to Support the New Safety Assessment Paradigm – Providing a Quantitative Bridge from *In Vitro* Concentration to Human Exposure in a Tiered Manner

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With a global paradigm shift occurring in toxicity testing towards the use of mechanistically based *in vitro* based testing and computational methods to assess the risks of chemicals, tiered testing strategies are being proposed and implemented to strategically use *in vitro* and *in silico* approaches in safety assessment. The focus of this presentation is on describing the necessary translation process referred as quantitative *in vitro* to *in vivo* extrapolation (QIVIVE) – making the use of *in vitro* data on bioactive concentrations for risk-based decisions for regulatory use for different tiers of safety assessment from prioritization to safe exposure estimation. To implement this tiered testing approach, availability of pharmacokinetic modeling tools with differing degrees of complexity and flexibility is crucial. For higher tier decisions, the utility of PBPK modeling is in utilizing the model of action knowledge gained from *in vitro* for risk-based decisions within a mode of action and adverse outcome pathway (AOP) framework. Their physiological structure facilitates the incorporation of *in silico*- and *in vitro*-derived chemical-specific parameters in order to predict *in vivo* kinetic behaviors of the test chemicals. With the use of modern parameterization methods based on *in silico* and *in vitro* data, PBPK modeling will provide the quantitative bridge from the bioactive concentrations *in vitro* to the *in vivo* exposure conditions using the internal exposure at the target tissue as a linking point. Recent progresses in the area of the development of open source PBPK modelling platforms like Population Lifecourse Exposure-To-Health-Effects Model (PLETHEM) suite have promises to efficiently describe the exposure, dosimetry, and health effect outcome continuum using the PBPK modeling module as an integration point. By leveraging the big data/ computational prediction tools included in the platform for rapid parameterization of the model such as human physiological parameters, *in vitro* metabolism data, and *in silico* prediction models for tissue partitioning, the open source PBPK platform will increase the applicability of the PBPK models to support new safety assessment paradigm beyond its traditional use areas.

[1]*ScitoVation, LLC*

A High-Throughput Microfluidic Platform for 3D Renal Proximal Tubule Toxicity Modeling

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Renal toxicity remains a major issue in drug discovery and stresses the need for better predictive models. Here, we describe the development of a perfused renal proximal tubule cell (RPTC) model in Mimetas' OrganoPlates® to predict kidney toxicity. The OrganoPlate® is a microfluidic platform which enables high-throughput culture of boundary tissues in miniaturized organ models. In OrganoPlates®, extracellular matrix (ECM) gels can be freely patterned in microchambers using capillary pressure barriers, providing boundary-free channels for passive medium perfusion. The microfluidic channel dimensions not only allow solid tissue and barrier formation, but also the growth of perfused tubular epithelial vessel structures. The goal of developing a perfused RPTC model is to reconstruct viable and leak-tight boundaries for performing cytotoxicity, as well as transport and efficacy studies. Human RPTC (SA7K clone, Sigma) were grown against an ECM in a 3channel OrganoPlate®, yielding access to both the apical and basal side. Tightness of the boundary over several days was shown by diffusion of a dextran dye added to the lumen of the tubule. Addition of toxic compounds resulted in disruption of the barrier which could be monitored in time. The time point of loss of integrity corresponds with the concentration and the toxic effect of the compound. Furthermore, fluorescent transport assays showed functional transport activity of in- and efflux transporters. The 3D proximal tubules cultured in the OrganoPlate® are suitable for high-throughput toxicity screening (96 individual models/plate), trans-epithelial transport studies, and complex co-culture models to recreate an *in vivo*-like microenvironment.

[1]*Mimetas US Inc.*

Vision for U.S. Strategic Plan to Implement Alternative Toxicology Testing

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The United States achieved a major goal with the reform of the Toxic Substance Control Act (TSCA), which could usher in the wide use of alternative approaches within the US Environmental Protection Agency (EPA). The EPA has been tasked by the amended TSCA to produce a strategic plan by June 2018 that will reduce the use of vertebrate animals and promote alternative testing methods. The legislation specifically requires alternative techniques such as high-throughput screening, computational toxicology, and other methods endorsed by regulatory bodies such as the Interagency Coordinating Committee on Validation of Alternative Methods or the Organization for Economic Co-operation and Development to be used instead of animal tests. Some of these techniques are well established for endpoints like skin and eye corrosion or sensitization. It is now critical that consideration be given to other endpoints such as acute inhalation, which could prove to be largely beneficial to smaller chemical companies struggling to meet regulatory compliance. The amended TSCA doesn't explicitly mention the use of Integrated Approaches to Testing and Assessment (IATAs) or Adverse Outcome Pathways (AOPs), although these approaches are endorsed by the OECD and anticipated to be included. However, what weighted approach or defined role will IATA's and AOPs have within the strategic plan? How do these methods fit into the EPA's proposed regulations for prioritization and assessment? Our objective is to highlight areas the US strategic plan for chemical safety should incorporate to achieve a more extensive use of alternative testing within risk-based regulatory decisions.

[1]*Physicians Committee for Responsible Medicine*

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Poster Abstracts

Preclinical Innovation and Patient Safety: A Collaborative Approach to Supporting Innovative Science and Replacing Preclinical Animal Tests

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Preclinical testing is critical to safe and effective drug development, yet traditional approaches remain poor predictors of human health outcomes. Many modern approaches to assessing preclinical drug safety are available and others are in development. However, modern approaches are not regularly submitted to FDA, as law and policy have not been updated, and training opportunities must be developed. The Preclinical Innovation and Patient Safety (PIPS) initiative aims to ensure industry and agency scientists have access to the best available tools to meet regulatory needs. A select group of stakeholders met in 2017 to begin developingthe initiative and identify projects to modernize the field. This presentation will highlight stakeholder efforts and identify additional scientific, legislative, regulatory, policy and educational opportunities to advance human-focused approaches, such as cellular and computational testing. Collaborative projects that aim to support human-focused science will help drive innovation, advance the field, and contribute to the more timely delivery of safer and more effective medicines. Submitted for oral or poster presentation. Authors do not consider this abstract as fitting in to one of the special sessions.

[1]*Physicians Committee for Responsible Medicine*

An Open-Source IVIVE Workflow Integrating QSAR and PK Models

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Many chemicals in commerce lack safety information. Accurate estimates of *in vivo* toxicity for these chemicals are needed to inform decisions on safe handling and use, as well as accidental exposure responses. High throughput *in vitro* assays provide a rapid way to evaluate potential chemical toxicity, but dose metrics and bioavailability need to be incorporated to allow interpretation and application of these data for risk assessment. To address this need, we have developed an open-source *in vitro* to *in vivo* extrapolation (IVIVE) workflow incorporating pharmacokinetic (PK) models with differing complexities. This workflow allows prediction of external administered dose corresponding to a predefined plasma concentration derived from *in vitro* assay data, or estimation of plasma concentration following a given dose. We developed a set of quantitative structure–activity relationship (QSAR) models to provide PK model inputparameters such as fraction unbound to plasma proteins, Henry's constant, and partition coefficients. Evaluation of the QSAR models' performance yields R2 values of 0.742-0.861 compared to experimental measurements. For chemicals lacking experimental PK parameter data, the QSAR predictions may be generated as part of the IVIVE workflow. Two case studies using the workflows, one focusing on assays measuring estrogenic activity and the other focusing on developmental toxicity, demonstrate how they provide a fast and straightforward approach to IVIVE analysis. This project was funded with U.S. Federal funds from NIEHS/NIH/HHS under Contract HHSN273201500010C.

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Non-Animal Testing Approach to Address Biocompatibility Testing of Medical Devices Required by the United States Food and Drug Administration (US FDA)

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Starting in December 2015, personal lubricants must receive pre-market approval from the US FDA Center for Devices and Radiological Health (CDRH) in order to be sold in the US. Part of the testing battery for biocompatibility includes the *in vivo* Rabbit Vaginal Irritation (RVI) test. We have created an Industry Consortium comprised of personal lubricants manufacturers and are working collaboratively with stakeholders and the US FDA to develop an *in vitro* testing approach to substitute for the RVI. Our Validation Program will analyze paired *in vivo* (and/or clinical)-*in vitro* data for vaginal irritation utilizing commercially available human reconstructed vaginal tissue models. A prediction model will be proposed that can be used for the safety assessment of personal lubricants. Our Validation Program proposal has been accepted in the Incubator Phase of the US FDA Medical Device Development Tool (MDDT) Pilot Program and is currently ongoing.

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Multiple Alternative Eye Assays Evaluate the Hazard of a Diluted Cleaning Product

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In vitro assays for screening product formulas for eye hazards reduce animal use, and are preferred by regulatory agencies. An oxidizing cleaning product concentrate was re-evaluated to determine the dilution at which the product balances efficacy and safety. Previous *in vivo* studies, per OECD Guideline 405, noted severe eye effects at relatively low dilution, minor eye effects at higher dilution, and a steep response curve. Evaluating irritancy of many formulations using *in vivo* assays was untenable from an animal welfare perspective; therefore, *in vitro* assays were used. A diluted product was evaluated by Bovine Corneal Opacity and Permeability (BCOP) assay, Chorioallantoic Membrane Vascular Assay (CAMVA), and Porcine Corneal Opacity and Reversibility Assay (PorCORA). In BCOP, the dilution had mean scores of 5.00 for opacity and 0.013 for permeability, and an IVIS of 5.20. In CAMVA, RC50 was 3.0%. PorCORA revealed complete corneal clearance by day 7, indicating reversible eye irritation/non-corrosivity. Combined results indicate a mildly irritating formula. BCOP was the preferred assay, particularly for oxidizing products. Results demonstrate *in vitro* assays for eye irritation provide a strong alternative for products, eliminating animal testing while maintaining a high level of confidence and accurately evaluating eye hazard. Given increased regulatory acceptance, this approach will expand for product testing, especially where many formulations exist.

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Resolving Corrosive/Severe Irritant Ocular Classifications Using an Alternative Dual Ex VivoAssay System

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Classifying severe ocular irritants vs. corrosives is traditionally determined by assessing reversibility of eye damage over time in rabbits (Draize Test). Currently, there are no alternative assays capable of assessing reversibility (healing). The Bovine Corneal Opacity and Permeability (BCOP) assay predicts corrosive classification if the IVIS is >55; but over-classifies materials causing reversible damage. .We demonstrate a dual-assay to distinguish corrosive from moderately/severely irritating materials. Twenty-one chemicals with known EPA ocular toxicity classifications were evaluated using BCOP. Chemicals with IVIS ≥20 were considered moderately irritating to corrosive and were further assayed by Porcine Cornea Opacity Reversibility Assay (PorCORA). PorCORA uses excised porcine corneas cultured for up to 21 days. Chemicals were topically dosed on the corneal epithelium and assessed for damage by fluorescein stain retention, as in Draize. Test chemicals causing stain retention through Day 21 were deemed corrosive (EPA Cat.1). If the damage cleared before Day 21, the test chemical was classified a severe/moderate irritant (EPA Cat.2/3). Of 21 chemicals tested, 6/6 Category 1 chemicals induced irreversible damage in PorCORA; damage caused by 15/15 Category 2 and Category 3 (IVIS ≥20-55) chemicals completely reversed by Day 21. This dual *ex vivo* assay fulfills an unmet need under current ocular hazard regulatory test guidelines, providing a non-live-animal (ex vivo) approach to differentiate between corrosive and severely irritating chemicals.

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Potency Ranking Dermal Sensitizing Chemicals Using the IVSA and epiCS® Skin Tissues

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Human 3D reconstructed skin epidermal equivalents release IL-18 in response to dermal sensitizing chemicals. Chemical concentrations producing positive responses greater than threshold (Stimulation Index, SI ≥2.0) correlates to potency or strength in an *In Vitro* Sensitization Assay (IVSA). Here 4-Nitrobenzylbromide (NBB) and DNCB strongly induced IL-18 secretion into culture medium (EC2.0=0.028% and 0.03%, respectively). Isoeugenol (IE) and Cinnamaldehyde (CA) were moderate sensitizers, while Resorcinol (RES, EC2.0=2.5%) and Hexylcinnamaldehyde (HCA, EC2.0=22%) were weak sensitizers. Potency rank was: NBB>DNCB>PPD,IE≈CA>RES>HCA, with NBB, DNCB and PPD classified as strong, IE and CA as moderate, and RES and HCA as weak sensitizers. Of 20 chemicals tested, seven were irritants, two were non-sensititzers (Glycerol, Isopropanol); Chlorobenzene (50%) was incorrectly predicted as very weakly sensitizing. Including all chemicals where epiCS® tissues viability was 10%-100% (via MTT assay), a cutoff SI ≥1.8 gave Accuracy of 95% and Sensitivity of 100%; Specificity, Negative and Positive Predictivity were >90%. We compared all data regardless of low viability (< 10%), and SI cutoffs from 1.6 to 2.0 in contingency tables. In summation, measuring IL-18 release from 3D tissues allows highly accurate, sensitive identification of dermal sensitizers. Also, the ability to rank-order potency of these chemicals based on EC1.8-EC2.0 values of IL-18 secretion is a powerful tool for further classification into potency categories.

[1]MB Research Laboratories, [2]CellSystems® Biotechnologie Vertrieb GmbH

Development of a Donor-Specific Endothelial Colony Forming Cell Platform to Assess the Variability of Human Responses to Drugs and Environmental Toxicants

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The variability of human responses to drugs and environmental toxicants cannot be assessed in animal models. Since direct testing in humans is unethical, addressing the intrinsic variability of human responses is a principal limitation of current toxicological assessments. However, this issue can be addressed using donor-specific primary cells isolated from blood or other tissues. The specific toxicity to be assessed depends on the type of cells employed. For example, cord or peripheral blood-derived endothelial progenitor cells, a.k.a. endothelial colony forming cells (ECFCs), can be used as a model in a population-wide screening platform for drugs and toxicants affecting human development and vascular function. We have tested the effects of several toxicants on proliferation of a panel of donor-specific ECFCs. Here, we demonstrate that ECFCs proliferation is a sensitive marker of response to a wide variety of environmental hazards, including metalorganic compounds and endocrine disruptors. Using several donor-specific cell lines, we show the variability in response to these agents in 384-well format. We further analyze the effects of toxicants in a 384-well functional anti-angiogenesis AA assay using ECFCs stably expressing mCherry red fluorescent protein. Thus, we demonstrate that donor-specific characteristics can be assessed at the cellular level. The development of this approach will contribute to the advancement of population-wide toxicological assessments.

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An Evaluation of Selected (Q)SARs/Expert Systems for the Prediction of Skin Sensitization Potential

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Predictive testing to characterize substances for their skin sensitization potential has historically been based on animal models such as the Local Lymph Node Assay (LLNA) and the Guinea Pig Maximization Test (GPMT). In recent years, EU regulations have provided a strong incentive to develop non-animal alternatives – both *in vitro* and *in silico*. Here we selected three different types of expert systems: Derek Nexus (knowledge based), TIMES-SS (hybrid), and VEGA (statistical), and evaluated their performance using two large sets of animal data, one of 1249 substances from eChemportal (354 sensitizers and 895 non-sensitizers) and a second of 515 substances curated by NICEATM (329 sensitizers and 186 non-sensitizers). We considered a model to be successful at predicting skin sensitization if it had at least the same balanced accuracy as the LLNA and the GPMT had in predicting the outcomes of one another, which ranged from 79% to 86% depending on the dataset. We found that none of the expert systems evaluated was able to achieve such a high balanced accuracy in their global predictions, with balanced accuracies ranging from 56% to 65%. However, for substances within the domain of TIMES-SS, balanced accuracies were found to be 79% and 82% for the 2 datasets respectively, in line with the animal data. While no model performed as well as the animal skin sensitization tests globally, compounds within the domain of TIMES-SS were predicted with the same balanced accuracy as the animal results. This abstract may not reflect U.S. EPA policy.

[1]*National Center for Computational Toxicology*

In Silico Prediction of Acute Oral Rat Toxicity

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Assessing the acute toxic potential of a substance is necessary to determine the potential effects of accidental or deliberate short-term exposure. There are no accepted *in vitro* approaches available and few *in silico* models to predict acute toxicity. Until recently, a paucity of experimental *in vivo* acute toxicity data was available for model development and evaluation. Here a large dataset of acute oral toxicity was compiled from different sources including the Hazardous Substances Databank (HSDB). Many of the studies were limit tests which report a LD50 as above a threshold, typically 2000 mg/kg or 5000 mg/kg. These data present challenges for model development because they give us less information than a LD50 value.To overcome this limitation, a two-step approach was used to model acute oral toxicity. In the first step, a random forest model was built to predict which substances would be above and below a LD50 of 5000 mg/kg. This model was constructed with a training set of 5931 substances with experimental LD50 values and ToxCast/Tox21 activities as descriptors. On a test set of 1482 substances, the balanced accuracy of the model was 76% and its negative predictive value was 84%. In the second step, a ridge regression model was derived using a training set of 4164 substances with experimental LD50 values and ToxCast/Tox21 activities as descriptors. For a test set of 1387 substances, 85% of the predictions made were within 1 log unit of their experimentally reported LD50 value. These models show considerable promise in predicting acute oral toxicity. This abstract does not reflect EPA policy.

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Characterization of Two Lung Cell Lines for Use in Cell Division Focused, Single-Cell Toxicity Assays

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The demand for predictive, effective, and realistic toxicology is at an all time high; specifically, cellular and computational toxicity research can advance our understanding about chemical exposure on living organisms without the need for whole animal testing. To meet this imperative change, we developed a new, cell division focused, single-cell toxicity approach. Previous research on silver nanoparticle exposure in retinal epithelial cells showed our assays to be insightful and practical. Here, we conducted a characterization study on two lung cell lines (16HBE and A549) that are broadly used in toxicology research to determine which would be most suitable for *in vitro* respiratory toxicity research. The cell line of choice will be used to investigate if and how chemicals affect cell division in a cellular lung model; thus, the ideal experimental system would be a cell line that performs cell division accurately and whose behavior resembles that of cells *in vivo*. We quantified mitotic timing, mitotic defects, chromosome number, and anchorage-independent growth. Both cell lines were capable of anchorage-independent growth. Moreover, the 16HBE cells displayed extended timing and gross mitotic defects, whereas A549 displayed normal timing and rates of mitotic abnormalities; however, both A549 and 16HBE were aneuploid (hypotriploidand hypodiploid, respectively). It should be noted that abnormal chromosome numbers produce altered transcriptomic and proteomic profiles, which in turn result in atypical cell behaviors. This study highlights fundamental issues of cell lines used in current toxicity studies and calls for caution in selection of experimental systems for *in vitro* toxicology.

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Understanding Global Acute System Toxicity Testing Requirements as a Framework for Rapid and Harmonized Acceptance of Alternative Approaches

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In order to move forward with replacing *in vivo* testing with nonanimal or alternative approaches, global acute systemic toxicity testing requirements need to be assessed. This is an imperative first step not only to clarify test requirements for each regulatory sector but also to understand how *in vivo* data is used by regulatory agencies so that alternative methods and strategies meet scientific and legal needs. Furthermore, companies, when exporting products, are currently required to comply with global regulatory legislation that could be different from their regional safety regulatory legislation. As replacement strategies become more widely accepted, duplicative testing to meet different regulatory requirements may become even more of a concern. A clear understanding of these requirements and potential hurdles will allow more harmonized acceptance of test methods and strategies. Therefore the International Council for Animal Protection in OECD Programmes (ICAPO), in collaboration with the United States and the European Commission, created a survey to catalog acute systemic toxicity requirements for OECD member countries. This data will be presented along with additional efforts to catalog non-OECD-member countries. Through collation of these data we aim to identify areas of potential international harmonization, improve understanding as to where development of alternative approaches is most urgently needed, and identify available scientific data that could increase our understanding of mechanisms of acute toxicity. Ultimately this information will help to shape strategies to replace *in vivo* acute systemic toxicity tests in the United States and speed harmonization of reduction and replacement strategies internationally.

[1]*Physicians Committee for Responsible Medicine*

Development of Human iPS Cell-Derived Mature Cardiomyocytes for Cardiotoxicity Assessment

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Drug-induced QT prolongation and proarrhythmia have been a major reason for drug withdrawal at late stage of clinical trials. Human induced pluripotent stem cell-derived cardiomyocytes (iPS-CMs) are expected to provide a new clue to assess the drug-induced proarrhythmic risk. Here we report a new in silico modeling of iPS-CMs for drug development. Our patch-clamp recordings revealed that iPS-CMs have spontaneous action potentials (APs) with relatively depolarized maximum diastolic potential (MDP; -50mV), suggesting an immature differentiation state of the iPS-CMs. To understand the mechanism by which iPS-CMs show immature property, we developed in silico modeling using iPS-CMs. Based on O'Hara-Rudy model, which is a gold standard of human ventricular AP model, we found that reduction of KCNJ2 caused automaticity and depolarized MDP in iPS-CMs. We next examined the effect of KCNJ2 using iPS-CMs. Adenoviral transduction KCNJ2 significantly hyperpolarized MDP (-70mV) and increased upstroke of APs (dV/dtmax) in iPS-CMs, suggesting that KCNJ2 is a functional maturation factor in iPS-CMs. Thus, our dry and wet experiments suggest that KCNJ2 plays a key role in iPSC-CMs. Our strategy would provide clues to safety assessment using iPS-CMs. This research is supported by grant from Japan Agency for Medical Research and development (#15mk0104053h0103 to YK).

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Using Artificial Intelligence and High-throughput Hormone Measurements to Predict Chemical Effects on Steroidogenesis

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We have constructed a Bayesian network to predict which enzymes in the steroidogenesis pathway may be impacted by chemical exposure. Briefly, the steroidogenesis pathway was represented as a network wherein steroid hormones and enzymes were defined as nodes, while edges between nodes represented conditional probabilities. For instance, two nodes lead into progesterone (HSD3B1 enzyme and the substrate, pregnenolone);and the probability ofprogesterone production is conditional on probabilities for both presence of pregnenolone and activity of HSD3B1. Using this network, we can apply Bayesian statistics to query the likelihood of any represented enzyme in the steroidogenesis pathway being active or inactive. Data for hormone levels were retrieved from the single concentration screening phase of the ToxCast high-throughput H295R assay for input. In this dataset, where 936 chemicals altered the levels of ≥1 hormone and a subset of 227 chemicals altered the levels of ≥4 hormones, our Bayesian network model predicted specific enzyme inhibition for 178 chemicals, identifying some novel targets for these chemicals and possible mechanisms underlying steroidogenesis disruption.

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Biologically-based Bayesian Networks and Application to Chemical Carcinogenesis

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Bayesian Networks (BN) provide a probabilistic means to predict an outcome based on observed data. This approach can be applied to predicting *in vivo* effects based on responses to high throughput screening (HTS) *in vitro* tests such as those from Tox21/ToxCast. We compiled pathway information from GO and KEGG databases about genes linkages with various hallmarks of cancer, such as angiogenesis, limitless cell replication, or evasion of apoptosis. These genes were cross-referenced with Tox21/ToxCast assays, and those assays were used to create *in vitro* summary scores for each cancer hallmark, for each chemical. *In vivo* carcinogenicity scores were based on proliferative pathology and neoplastic lesion endpoints from the U.S. EPA's ToxRefDB collection of rat and mouse cancer studies. A structure-based mutagenicity predictor was obtained for each chemical using the EPA's Toxicity Estimation Software Tool. The overlapping chemicals tested in both Tox21/ToxCast and ToxRefDB were used to form the training data. Multiple networks were created through both data-driven and network-learning approaches, and used to predict the carcinogenicity of chemicals based on the hallmark summary scores and mutagenicity features. The machine-learned networks outperformed the data-driven models, and the best model resulted in 82% accuracy overall. The model showed high positive predictivity, indicating that chemicals that perturb many hallmark-related assays are more likely to cause cancer in rodent studies. This work supports the idea that it is possible to correlate *in vitro* bioactivity response patterns of certain chemicals and their carcinogenic potential *in vivo*, but highlights difficulties in predicting such biologically complex endpoints.

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Use of Threshold of Toxicological Concern (TTC) with High Throughput Exposure Predictions as a Risk-Based Screening Approach to Prioritize More Than Seven Thousand Chemicals

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A risk-based prioritization approach using the Threshold of Toxicological Concern (TTC) combined with high-throughput exposure (HTE) modelling is presented. We started with 7968 chemicals with previously calculated population median total daily exposures characterized by an upper 95% credible interval (UCI). Substances were profiled using the TTC workflow of Kroes et al (2004) taking into account the known TTC exclusions and structural alerts. UCI total daily exposures were compared to the appropriate class-specific TTC. For Cramer Class I, 0 of 1294 substances had UCIs greater than the TTC; for Cramer Class II 0 of 332 had UCIs above the TTC; for Cramer Class III 58 of 3214 UCIs were greater than the TTC; and for cholinesterase inhibitors 1 of 102 had a UCI above the TTC. For the 1853 chemicals with genotoxicity structural alerts, modeled UCI exposures for the vast majority exceeded the TTC of 0.15 μg/day (using median exposure values, only 79 were above the TTC). Using the ICH (2014) TTC value for mutagenic impurities of 1.5 μg/day (corresponding to an individual excess lifetime cancer risk of 1x10-5), the UCI exposure values of 333 substances were greater than this TTC (using the median exposure values 19 were above the ICHTTC). For substances that exceed TTCs, we discuss options for subsequent evaluation depending on the decision context. Overall, this analysis indicates that TTC and HTE are potentially useful as a pragmatic first step ina risk-based prioritization approach for chemical safety evaluations. This abstract does not reflect EPA policy.

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Evaluating Alternative Toxicity Testing Approaches for Food Relevant Chemicals

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Alternativetoxicity testing methods have been developed to screen thousands of chemicals in an efficient manner and reduce the need for animal testing. Examplesinclude data from efforts such as high-throughput screening (HTS) Tox21/ ToxCast programs and read-across tools such as the Organization for Economic and Development toolbox. In this study, we compared the results from traditional toxicity studies with predictions from these alternative testing methods for food relevant chemicals in ToxCast. Results from *in silico* estrogen receptor (ER) and androgen receptor (AR)models, which integrate HTS from ToxCast/ Tox21, as well as outputs from read-across tools were used to evaluate food relevant chemicals. We identified 89 putatively endocrine active, non-cytotoxic chemicals. These 89 chemicals were further filtered based on the availability of *in vivo* data related to developmental and reproductive toxicology (DART). This resulted in 10 chemicals: 3 ER agonists, 1 ER antagonist, 1 AR agonist, and 9 AR antagonists. Read-across methods were used to identify potential analogues for each target chemical. Using structural similarity and similar mode of actionas our primary criteria, 9 target chemicals were identified for which the analogue approach was used to predict their ability to elicit *in vivo* effects related to DART. The pattern and potency of the analogues identified using read-across approaches were compared to the known *in vivo* DART effects foreach target chemical. This study demonstrates that HTS data could be used to prioritize chemicals and read-across approaches further build weight of evidence for studying chemicals from a food safety perspective. This abstract does not necessarily reflect EPA policy.

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Quality Considerations: Redefining Test Systems from Animals to Tissues and Beyond

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The use of non-whole animal test methods transforms the way regulatory requirements are applied in preclinical testing. Recent global regulatory initiatives emphasize the importance of transitioning to human relevant assays and test systems that do not use animals. When these methods are moved from research into the regulated arena, GLP principles must be followed. The GLPs were originally written in the 1970s, when the vast majority of regulated research was performed using animals as the test system. Current innovative, alternative test systems include *ex vivo* tissues, manufactured biological systems, three dimensional tissue constructs, and cell cultures maintained in dynamic flow bioreactors. Each type of alternative test system raises new quality and compliance points to consider when used within a regulatory context. Just as the applications of these methods have advanced with regulatory acceptance, the quality control and compliance of these test systems must also progress.

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ASCCT 2017 Awards

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 Toxicity 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. The first award winner was Dr. Nicole Kleinstreuer for her poster Identifying Reference Chemicals for Androgen Receptor Activity.

In 2016, we were honored to host the wife and brother of the late Dr. Edward Carney to award the 2nd annual Ed Carney Award for Predictive Toxicology, which went to Emma Bowers for her presentation Modeling a complex *in vivo* response *in vitro*: Exploring heterogeneity and mechanisms associated with ozone adaptation.

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. In 2016, the award went to Ellen Garcia for her presentation Single-cell analysis reveals that silver nanoparticle exposure leads to multi-nucleation through defective cell division.

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