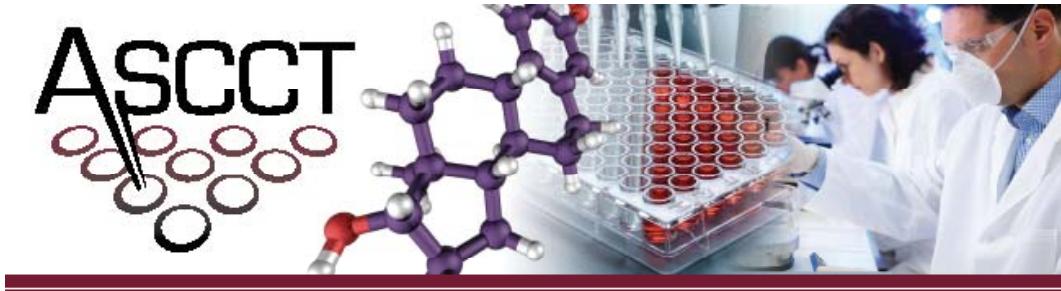

3rd Annual Meeting of the American Society for Cellular & Computational Toxicology

November 12, 2014
Lister Hill Auditorium, NLM, NIH
Bethesda, MD

ASCCT

American Society for
Cellular and Computational
Toxicology

www.ascctox.org

President's Welcome

Welcome everyone to the 3rd annual meeting of the American Society for Cellular and Computational Toxicology! Last year's meeting showed significant growth in attendance from our first meeting, and I'm sure that this year's meeting will once again set a record. Hopefully my appearance last year in a Halloween mask hasn't deterred any faint hearted folks from showing up. …and I promise not to engage in any completely non-professional behavior this year!

I especially wouldn't want to spoil any portion of the exciting program your Board of Directors and the organizing committee have planned for today. We're focusing on Adverse Outcome Pathways (AOPs), and how these mechanistic constructs can be used in regulatory and investigational toxicology. We have a distinguished group of invited speakers who will be addressing various aspects of AOPs, and I invite all attendees to interact with our speakers as much as possible – not only during the planned discussion session, but at the lunch and breaks as well.

Many of you have also expended the time and energy to share your research efforts by preparing posters. I want to encourage all attendees to show their appreciation by taking advantage of the specified poster viewing times shown in the Agenda. Interactions at these poster sessions should be an integral part attending this annual meeting.

As many of you know, we signed a Memorandum of Understanding last year with a sister organization – The Japanese Society for Alternatives to Animal Experiments (JSAAE). Two members from JSAAE attended our meeting last year, and I was invited to the 26th annual meeting of their society last December. This year the ASCCT is hosting Dr. Takashi Yamada from JSAAE and the Japanese National Institute of Technology and Evaluation. Dr. Yamada will present on a tool for predicting repeat dose toxicity. Please help to make our guest feel welcome in the Washington DC area.

While I'm discussing today's annual meeting, let me make sure to give all the credit to the annual meeting organizing committee who have put together this fine program. Please thank Arya Birdie, Suzanne Fitzpatrick, Jack Fowle, Erin Hill, Miriam Mossoba and Kristie Sullivan for putting forward their time and effort in designing today's activities. While you're at it, you might give Kristie a few extra kudos for her work in "producing" our much admired webinar program.

Today's meeting is also an opportunity for all ASCCT members to select their Board of Directors. It's time for me to step down from the Board, even though it has been an immense privilege to serve for four years as your first president. Certainly serving the last two years with our current fantastic Board (Marilyn Aardema, Jack Fowle, Marianna Gaça, Thomas Hartung, Erin Hill, and Kristie Sullivan) has been a real pleasure, just as it's been a real pleasure to watch the ASCCT grow from the original three folks (Kristie, Erin and me) who founded the organization, to the over 170 individuals who are now members. …and these numbers will continue to grow, I'm absolutely certain, since the future of toxicology lies in exactly our areas of expertise.

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It is the *in vitro* cellular methods and the computational approaches which will be the focal point of toxicology for the next decades. Let's enjoy the changes!

Even though I think ASCCT membership is its own reward, I want to thank - and congratulate - each of you for becoming ASCCT members. Many of you have convinced your company management to be financially supportive as well, and I'd like to encourage others to do the same. We're still operating on a pretty thin margin, and there are many more things we can do to benefit our members if our finances allowed. Speaking of support, I should now take the time to thank the organizations whose contributions have made much of this annual meeting possible – Alternatives Research and Development Foundation, AltTox.org, British American Tobacco, CAAT, IIVS, Physician's Committee for Responsible Medicine, and PETA International Science Consortium, Ltd.

Finally – just as I did last year and the year before - I want to urge all of you to take the opportunity at this meeting to introduce yourself to someone you haven't met before, and initiate the scientific networking that is so important to each of our professional successes. Our two poster viewing sessions would be a great time to do this! The ASCCT was envisioned as a platform where regulatory and investigational scientists from both the computational and cellular sides of toxicology could freely exchange ideas. Please do it!!

Your president,
Dr. Rodger Curren

ASCCT Board of Directors
Rodger Curren, President
Erin Hill, Treasurer
Kristie Sullivan, Secretary
Marilyn Aardema
Jack Fowle
Marianna Gaca
Thomas Hartung

Annual Meeting Organizing Committee
Arya Birdie
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Jack Fowle
Erin Hill
Miriam Mossoba
Kristie Sullivan

Notes

Table of Contents

ASCCT Would Like to Thank the Following Meeting Sponsors

Alternatives Research & Development Foundation

AltTox.org

British American Tobacco

CAAT Johns Hopkins University Center for Alternatives to Animal Testing

IIVS Institute for In Vitro Sciences Advancing Science & Animal Welfare Together

PCRM Physicians Committee for Responsible Medicine

PETA International Science Consortium, Ltd.

Notes

ASCCT

The William and Eleanor Cave Award

The William and Eleanor Cave Award is presented to recognize achievements in developing alternatives to the traditional use of animals in testing, research or education.

It is presented biannually by the Alternatives Research & Development Foundation and carries a $5,000 prize.

Past recipients (and their affiliations at the time of the award) have included:

- Ruy Tchao, University of the Sciences
- George Russell, Adelphi University
- John Sheasgreen, MatTek Corporation
- Leon Bruner, The Gillette Company
- Daniel Smeak, The Ohio State University
- Rodger Curren, Institute for In Vitro Sciences
- Mel Andersen, The Hamner Institute for Health Sciences

A special award was presented in 2010 to the journal ATLA (Alternatives To Laboratory Animals), for its invaluable contributions to advancing the science of alternatives.

William and Eleanor Cave were devoted officers of The American Anti-Vivisection Society for decades. They recognized the opportunities in developing new technologies and alternative methods to address the problems of animal experimentation. They dedicated resources to fund research, eventually resulting in the establishment of the Alternatives Research & Development Foundation.

ARDF's mission is to fund and promote the development, validation and adoption of non-animal methods in biomedical research, product testing and education. ARDF has awarded 2.8 million dollars in grants to researchers developing alternative test methods at major universities across the U.S. and sponsors scientific meetings such as the World Congresses on Alternatives and Animal Use in the Life Sciences. Information is available at www.ardf-online.org.

Alternatives Research & Development
FOUNDATION

Where Chemistry and Biology Meet: AOPs as a Framework for Advancing Toxicology
3rd Annual Meeting of the
American Society of Cellular and Computational Toxicology

ASCCT

11/12/2014

Lister Hill Auditorium, NLM, NIH
Bethesda, Md.

8:30 – 8:45	**Welcome**	Rodger Curren, ASCCT President Institute for In Vitro Sciences
8:45 – 9:30	**Adverse Outcome Pathways: From Scientific Discovery to Regulatory Decision-Making**	Robert Kavlock, ORD, Environmental Protection Agency
9:30 – 10:15	**Building Pathways in Health Research: NIH's Big Data to Knowledge Initiative and Library of Integrated Network-Based Cellular Signatures (LINCS) Project**	Jennie Larkin, National Heart Lung and Blood Institute, NIH & Ajay Pillai, National Human Genome Research Institute, NIH
10:15 – 10:30	**BREAK**	
10:30 – 11:00	**Development of New Computational Approaches based on AOPs for Liver Toxicity**	Stephen Enoch Liverpool John Moores University
11:00 – 12:00	**Panel & Audience Discussion**	Robert Kavlock Stephen Enoch Jennie Larkin Ajay Pillai Louis "Gino" Scarano, OCSPP, EPA
12:00 – 1:30	**Lunch and Poster Viewing**	
1:30 – 1:50	**Predicting Neurotoxicity in Human-Derived iPSC 3D Mini-Brains**	David Pamies, Johns Hopkins University, CAAT
1:50-2:10	**Predicting Acute Toxicity using In Vitro ToxCast™ HTS Mitochondrial Inhibition Assays**	Barun Bhhatarai, Dow Chemical
2:10-2:30	**Comparative Use of the Scientific Confidence Framework for Adverse Outcome Pathways to Assess Potential Applications in Regulatory Decision-Making**	Katy Goyak, ExxonMobil
2:30-2:50	**Activities Aimed at Harmonizing the International Implementation of Test Methods or Approaches that Accomplish the 3Rs**	Mei-Chun Lai, PCRM
3:00 – 3:30	**Break and Poster Viewing**	
3:30 – 4:00	***Coordination with International Societies: Japanese Society for Alternatives to Animal Experiments*** **HESS: A Tool for Predicting Repeated Dose Toxicity with Toxicological Categories Based on Adverse Outcome Pathways**	Takashi Yamada, National Institute of Technolgoy and Evaluation (NITE), Japan and member of the Japanese Society for Alternatives to Animal Experiments
4:00 – 4:15	**World Congress on Alternatives and Animals in the Life Sciences: Report from Prague and Looking ahead to Seattle**	JoAnn Zurlo, CAAT
4:15 – 5:00	**ASCCT Business meeting**	ASCCT Officers
5:00 – 6:00	**Reception**	Presentation of the Cave Award by the Alternatives Research and Development Foundation

The American Society for Cellular and Computational Toxicology (ASCCT)

Mission:

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

Goals:

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

All Members will receive:

- Quarterly e-newsletter
- 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**

Poster Abstracts

Supplemental Information

Assessing Increased Sensitivity and Variability Issues in an Established In Vitro Phototoxicity Testing Program

Sparks, Jessica, Krawiec, Lindsay; Hilberer, Allison

Institute for In Vitro Sciences, Inc.
Gaithersburg, MD, USA

The 3T3 Phototoxicity assay is an established *in vitro* assay used to evaluate the potential phototoxicity hazard of a test chemical. The assay was developed and validated by ZEBET. In 2004, the Organization for Economic Cooperation and Development (OECD) formally recognized the 3T3 Phototoxicity assay through the establishment of OECD Test Guideline (TG) 432. IIVS has performed the 3T3 Phototoxicity assay since 1995 and has maintained a historical database of positive control test results which are used to establish assay acceptance criteria. The evaluation of assay performance during routine testing is based on the comparison of the positive control and solvent control results to our historical data. When the assay is not consistently meeting acceptance criteria, the results necessitate a closer examination of assay performance. Recently, the 3T3 Phototoxicity assays at IIVS have resulted in invalid trials due to 1) high assay sensitivity and 2) low optical density signals. The focus of our R&D work was to examine the storage conditions, preparation, and manufacturer's lot-to-lot consistency of reagents (DMSO, neutral red, and chlorpromazine) and to investigate our light source (Dermalight SOL 3 Solar Simulator) for impacts on irradiance uniformity, cell sensitivity, and consistency over time. We observed differences between the type (i.e. catalog number) and lot of DMSO used to prepare chlorpromazine and solvent controls. Additionally, we determined that preparation, storage conditions, and filtration methods of neutral red can affect the optical density signal. We measured variations in the UV light intensity under the light source, and recognized that the likelihood for variations in dose or solvent control responses was dependent on plate positioning under the light. Through this work, we identified several variables which affected assay performance and contributed to increasing assay sensitivity. The results of this work provided an understanding of the impacts of assay-specific reagents, supplies, and equipment which will be used to continually assess and optimize assay performance.

Comparison of Cramer Class Determination between Toxtree, OECD QSAR Toolbox and Expert Judgment

Sneha Bhatia[1], Terry Schultz[2], David Roberts[3], Jie Shen[1], Lambros Kromidas[1], and Anne Marie Api[1]

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Woodcliff Lake, NJ, USA
[2]The University of Tennessee, College of Veterinary
Knoxville, TN, USA
[3]Liverpool John Moores University
Liverpool, UK

The Threshold of Toxicological Concern (TTC) is a pragmatic approach in risk assessment. The Cramer decision tree is used extensively for determining a safe exposure threshold by classifying chemicals into three different classes. Assigning an accurate Cramer class to a material is a crucial step to preserve the integrity of the risk assessment. In this study the Cramer class of over 1000 fragrance materials across diverse chemical classes, were determined by using Toxtree (TT), OECD QSAR Toolbox (TB), and expert judgment. Disconcordance was observed between TT and TB. A total of 165 materials (16%) showed different results from the two programs. The overall concordance for Cramer classification between TT and expert judgment is 83%, while the concordance between TB and expert judgment is 77%. Additional analyses were conducted to determine the concordance within various chemical classes. Strategies and guidance on determining the Cramer class for various chemical classes are discussed.

Predicting Acute Toxicity using In Vitro ToxCast™ HTS Mitochondrial Inhibition Assays

Barun Bhhatarai, Dan Wilson, Paul Price, Mike Bartels, Shubhra Chaudhuri and Ed Carney

Toxicology Environmental Research and Consulting, The Dow Chemical Company
Midland MI, USA

Mitochondrial inhibition is a mechanism known to drive acute toxicity for certain chemicals. High Throughput Screening (HTS) assays have been developed to test if a chemical's toxicity operates by this mechanism. We hypothesized that, for chemicals that cause mitochondrial inhibition in HTS assays, acute toxicity is conserved across invertebrate, aquatic and mammalian species, suggesting that
1) In the absence of pre-systemic metabolism or limited absorption, *in vitro* mechanistic data could predict responses in multiple species,
2) Under conditions of similar bioavailability, concordance of dose response between species would be high, and
3) Predictions of oral toxicity from HTS assays routes would often be confounded by chemical-specific differences in uptake and metabolism.
To validate our hypothesis, we determined whether,
1) *In vitro* data for mitochondrial inhibition generated by the US-EPA ToxCast™ program was correlated with various measures of acute toxicity,
2) Read-across could be used between Rat, Daphnia and Fish acute toxicity for those substances that cause mitochondrial inhibition in *in vitro* systems, and
3) Incorporation of predictions of gastro-intestinal absorption and first pass metabolism improved correlations between acute toxicity observed by the oral and intravenous routes.

We observed that,
1) Mitochondrial inhibition predicted the minimum toxicity (an upper bound to the LC_{50}'s) of the chemicals in Fish and Daphnia. Chemicals could cause higher toxicity by other mechanism but never lower. The lower the assay AC_{50} the more likely the toxicity was driven by mitochondrial toxicity. Therefore the assay can be used to set a minimum toxicity for chemicals and low AC_{50} values mean high Fish and Daphnia toxicity.
2) Mitochondrial inhibition didn't predict the toxicity of the chemicals in Rat with limited oral bioavailability and first pass metabolism, which renders compounds much less toxic. Simulations using *in silico* models of bioavailability and metabolism (for three Cyp enzyme s -2C, 2D and 3A; and substrates for UDP-glucuronosyltransferases -UGTs) improved toxicity correlations. Predictive models for non-Cyp phase 1 and 2 metabolism are currently not available and would likely provide further insight.
3) Consistent with the AOP approach, such predictions could reduce the need for nonclinical regulatory safety testing for acute toxicity.

Preliminary Investigation on Reducing Ocular Irritation Potential of Harsh Ingredients by Increasing Formulation Viscosity

Elizabeth A. Sly and Kimberly Norman

Institute for In Vitro Sciences, Inc.
Gaithersburg, MD, USA

Formulations tested for ocular irritation using the Bovine Corneal Opacity and Permeability (BCOP) assay may be assigned a specific irritation label based on the resulting In Vitro score and specific regulatory guidelines (e.g. OECD, EPA, and CLP) that provide cutoff values for classifications. The ability to reduce ocular irritation by slightly adjusting the physical properties of a formulation is highly desirable. Laboratory investigations found that incrementally changing viscosity using increasing amounts of Carbopol® as a thickening agent reduced ocular irritation when mixed with a 1% NaOH solution in water. Following a 10-minute exposure in the BCOP assay, 1% NaOH was previously classified as a severe ocular irritant (In Vitro score=161.6). Increasing Carbopol® from 0.25% to 1.25% in a mixture with 1% NaOH decreased the In Vitro score to a range of values between 150.9 and 18.3 and decreased ocular irritation across a range of irritation classifications from severe to mild irritation (n= 3 corneas per treatment). Exposure to 1% Carbopol® alone exhibits minimal irritation (In Vitro score=1.3) and Carbopol® is consequently not considered to contribute to ocular irritation within the tested mixtures. Histopathology evaluation further supports that exposure to 1% Carbopol® results in damage similar to negative control treated corneas and that epithelial and stromal damage decreases as viscosity increases. Additionally, preliminary findings indicate that when a small amounts of thickener is added to a complex formulation containing otherwise harsh ingredients, ocular irritation can be mitigated from a Category I label to a Category II label according to current EPA guidelines applicable to cleaning products making anti-microbial claims. Similarly, increasing the viscosity of a formulation containing more than 3% of a severe ingredient also resulted in a "Not Classified" label according to OECD criteria when it would have received a severe classification if left untested (according to CLP regulations). These results indicate that increasing viscosity may be an effective tool for reducing ocular irritation potential of a formulation. Viscosity, among other physical properties, may therefore be used to inform decision making during product development, ultimately affecting down stream users in such areas as marketing, labeling, packaging and distribution.

Benchmark Study: Structural Similarity Search Methods for Identifying Read-Across Analogs

Jie Shen

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As alternative approaches to fulfill data gaps in chemical safety assessment, in silico methods, such as read-across and quantitative structure activity relationship (QSAR), have generated much attention. Such approaches have been used in ECHA (European Chemicals Agency) submissions of chemical dossiers for REACH (Registration, Evaluation, Authorization and Restriction of Chemicals). Searching and identifying suitable structural analogs for the chemical of interest is prerequisite for read-across. Although several methodologies and programs are available for analog searching, there are limited practical strategies or guidance for conducting analog search. This work compared several popular analog search programs using 14 different materials reported in a published paper by Blackburn et.al.[1] For each material, 11 different analog search methods from 5 different programs were conducted. The top 100 and top 200 analogs with Tanimoto similarity[2] more than 0.5 were compared with the suitable analogs reported in Blackburn et al.'s paper.[1] The results showed that Pipeline Pilot with FCFP4 fingerprint outperforms other methods in most cases. For most of the materials, the analogs were enriched in Top 100 candidates. OECD QSAR Toolbox fails to identify analogs for nitro and amino contained materials.

References:
[1]Blackburn, K.; Bjerke, D.; Daston, G.; Felter, S.; Mahony, C.; Naciff, J.; Robison, S.; Wu, S., Case studies to test: A framework for using structural, reactivity, metabolic and physicochemical similarity to evaluate the suitability of analogs for SAR-based toxicological assessments. Regulatory Toxicology and Pharmacology 2011, 60 (1), 120-135.
[2]Rogers, D. J.; Tanimoto, T. T., A Computer Program for Classifying Plants. Science 1960, 132 (3434), 1115-1118.

Network Motifs that Recur Across Species can Inform Adverse Outcome Pathways

Robert Borotkanics and Harold Lehmann

Johns Hopkins Bloomberg School of Public Health, Johns Hopkins School of Medicine
Baltimore, MD, USA

Background
The NAS toxicity testing vision is grounded in the notion of toxicity-pathways. A toxicity pathway by definition is a pathway whose biological function is compromised when sufficiently perturbed, leading to toxicity and disease. Much effort has been devoted to definitions concepts and possible application of the toxicity-pathway concept; however, the concept has not really advanced over the past 7 years. We decided to take a step back and look at the concept more broadly. Rather than look for toxicity-pathways, or adverse outcome pathways, we decided instead to research intracellular networks more broadly and investigate if there exists particular unique patterns of biological interaction. This exploration bared fruit, resulting in a recent publication within the Archives of Toxicology, May 2014. We discovered that in intracellular networks, there are defined patterns of interactions, called motifs. Cellular molecules interact in complex ways, giving rise to a cell's functional outcomes. It has been learned that many of these interactions can be represented abstractly as a network and within a network there in many instances are network motifs. Network motifs are subgraphs that are statistically overrepresented within networks. To date, specific network motifs have been experimentally identified across various species and also within specific, intracellular networks; however, motifs that recur across species and major network types have not been systematically characterized. We reason that recurring network motifs could potentially have important implications and applications for toxicology and in particular, toxicity testing. Therefore, the goal of this study was to determine the set of intracellular, network motifs found to recur across species of both gene regulatory and protein-protein interaction networks.

Results
We report the recurrence of 13 intracellular, network motifs across species. Ten recurring motifs were found across both protein-protein interaction networks and gene regulatory networks. The significant pair motif was found to recur only in gene regulatory networks. The diamond and one way cycle reversible step motifs were found to recur only in protein-protein interaction networks.

Conclusion
This study is the first formal review of recurring, intracellular network motifs across species. Within toxicology, combining our understanding of recurring motifs with mechanism and mode of action knowledge could result in more robust and efficient toxicity testing models. We are sure our results will support research in applying network motifs to toxicity testing.

Non-Animal Testing: It's Within REACH

Amy J. Clippinger, Jeffrey Brown, Julia Baines, and Gilly Stoddart

PETA International Science Consortium, Ltd.,
London, UK

When the EU passed the REACH legislation, this testing programme was intended to ensure animals were used only as a last resort. Yet reports show that, so far, more than 800,000 animals have died in REACH tests with millions more expected to be used in the coming years. To minimise animal testing, REACH contains a number of specific measures and general provisions designed to establish and enforce the last resort principle. For example, non-testing methods such as read-across, weight-of-evidence approaches and QSARs, and non-animal testing methods must be used wherever possible.

Integrating and interpreting nonstandard information generated for key events within a rational framework can be used to provide an assessment of a toxicity endpoint that is more predictive of human health effects than testing on animals. Integrated Approaches to Testing and Assessment (IATA) provide opportunities for minimising the use of animals and promote the efficient use of resources. To be successful, an IATA should have a rational, knowledge-driven design, include both *in vitro* models and computational methods, and be based on a well described Adverse Outcome Pathway (AOP). IATA and testing strategies are now available or in development for many of the REACH Annex VII and VIII endpoints including skin sensitisation and skin and eye irritation and corrosion. However, the REACH Annexes and Test Method Regulation are lagging behind the latest developments in toxicology assessment, resulting in animals being used in testing that is not required by law. Here we outline specific methods which may be used to minimise the use of animals for REACH.

Collaboration Platform For Comprehensive Lung Biological Network Models For Toxicological Risk Assessment

Stephanie Boue[1], Anselmo Di Fabio[2], Brett Fields[3], William Hayes[3], Julia Hoeng[1], Jennifer Park[3], Manuel Peitsch[1], Walter Schlage[1]and Marja Talikka[1]

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Biological network models provide a framework to study adverse outcome pathways initiated by exposure to environmental chemicals and drugs. The comprehensiveness of these network models is important to capture all possible key downstream effectors linking chemical compounds to specific biological pathways. A current limitation in the use of biological network models is an easy way to update, collaborate and share these networks. We have created a web-based crowdsourcing platform to facilitate collaboration on 50 networks capturing a wide range of biological processes as part of the sbvIMPROVER Network Verification Challenge (NVC). These networks consist of causal statements in the Biological Expression Language (BEL) based on both literature and data, that capture a series of intermediate molecular steps that help explain potential pathways between the initiating molecular event to adverse outcome. The goal of the NVC is for the scientific community to vote, comment and add new biology to improve these networks. The first NVC resulted in the improvement of the networks with over 2000 votes cast and 800 literature evidences added to the website (http://bionet.sbvimprover.com). Networks continue to be improved during the second NVC (NVC2) with a long-term goal of making them the most up-to-date and comprehensive networks that can be used by the scientific community for toxicological risk assessment and drug development.

Predicting Neurotoxicity in Human-Derived iPSC 3D Mini-Brains

Pamies David[1], Block K[1], Smirnova L[1], Harris G[1], Bressler J[2], Palma-Lima M[2], Christian KM[3], Ce Zhang[3],Hartung T[1] and Hogberg HT[1]

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We have limited understanding of the function of the CNS and the complexity of the brain, especially during development and neuronal plasticity. Simple *in vitro* systems do not represent physiology and function of the brain. Development of 3D organoid systems has generated more complex *in vitro* human models that better simulate the organ's biology and function. The "human-on-a-chip" program is one of the most ambitious initiatives in toxicology initiated by NIH, FDA and DARPA. Our project funded by NCATS, NIH (1U18TR000547) is part of this program and aims to establish and characterize an *in vitro* model of the developing human brain for the purpose of testing drugs and chemicals. To accurately assess risk, a brain model needs to recapitulate the complex interactions between different types of glial cells and neurons in a 3D platform. Moreover, human cells are preferred over cells from rodents to eliminate cross-species differences in sensitivity to chemicals. The use of iPSC allows us to address gene environment interactions of different donors and makes it possible to evaluate inter-individual sensitivities to chemical exposure. The 3D model has shown to recapitulate early *in vivo* human neurodevelopment. Showing the emergence of different kinds of neurons and glial cells, induction of genes that play important roles in neurodevelopment as well as presence of active glutamate receptors by functional calcium live imaging. We have used Rotenone, a pesticide known to induce neurotoxicity by inhibition of mitochondrial complex I, in order to test the relevance of the model to predict toxicity. The model shows increased ROS production and decreased mitochondrial function after exposure to Rotenone. In addition, the model shows a decrease of sensitivity to rotenone exposures with increasing maturation. Eight week mini-brains exposed for 48 hours to 10µM Rotenone showed 25% decrease of mitochondrial activity while two week mini-brains exposed to the same concentration showed 80% decrease of mitochondrial activity. Notably, such human brain models will represent a versatile tool for more complex testing platforms and strategies as well as research into (developmental) neurotoxicity as well as CNS physiology and pathology.

Predicting the Eye Stinging Using the Novel NociOcular Assay

Victoria Diersen, Lindsay Krawiec, Elizabeth Sly, Kimberly Norman

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Several *in vitro* eye irritation models exist; however, no eye irritation models have demonstrated the ability to accurately predict eye stinging. The NociOcular assay, a novel neuronal model based on activation of the Transient Receptor Potential Vanilloid type 1 (TRPV1) channels, has been shown to distinguish stinging from non-stinging products. In the NociOcular assay, the TRPV1 channel expressing SH-SY5Y neuroblastoma cells are exposed to a serially-diluted test substance and TRPV1 channel activation is measured by acute increases in the intracellular free Ca^{2+}. Although the NociOcular assay was originally designed to predict the eye sting potential of surfactant ingredients and surfactant-based products, there are many other product types which may come in contact with the eyes, such as sunscreens. In this study, we sought to evaluate sunscreens and other products that are used near the eyes. We developed alternate solvents and a modified dilution method to overcome solubility and viscosity limitations and to more accurately model in-use exposures. Furthermore, we investigated the possibility that the alternate solvents and modified dilution method could affect the results of the assay. During proof-of-concept studies, the assay modifications allowed for greater solubility, and controls performed as expected. Additionally, using these modifications of the assay, we successfully measured TRPV1 channel activation caused by products which are hydrophobic, viscous, and may come into contact with the eyes at a high concentration. Future research will focus on evaluation of target ingredients in insoluble products and further modifications of the assay to assess final product formulations.

Comparative Use of the Scientific Confidence Framework for Adverse Outcome Pathways to Assess Potential Applications in Regulatory Decision-Making

Katy Goyak[1], Christine Palermo[1] and Grace Patlewicz[2]

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Newark, DE, USA

The adverse outcome pathway (AOP) concept is an organizing framework describing linkages between initial molecular events and adverse outcomes at the individual or population levels. In the 5 years since the concept was first introduced into the peer-reviewed literature, over 50 AOP articles have been published, with nearly half written prior to the release of OECD guidance on AOP development (June 2013) and all written prior to the most recently available supplement to the AOP guidance (Sept 2014), that identifies consideration of potential applications for an AOP as beneficial. This consideration was not listed earlier due in part to the difficulty in forecasting all potential regulatory applications. A literature survey was completed using the public launch of the AOP-Wiki (Sept 2014) and peer-reviewed publications (n=39 pathways; n=56 publications; as of Oct 1, 2014) to generate descriptive statistics on perceived AOP applications. Based on the AOP-Wiki, 5 proposed applications were identified: "test strategy development" (n=10 entries), "hazard assessment" (n=2), "knowledge gap identification" (n=2), "assay development" (n=1), and "risk assessment" (n=1). Applications were not addressed for approximately 60% of the AOPs in the AOP-Wiki. To assess whether a proposed application might be reasonable for an individual AOP, a comparative analysis of three case studies from the published literature was completed, using the recently proposed Scientific Confidence Framework (SCF) for AOPs. The three case studies (acetylcholinesterase inhibition leading to acute mortality (PMID24922588); drug-mediated bile salt export pump inhibition leading to cholestatic liver injury (PMID23945500); and categorization of repeated-dose toxicity of hair dyes (PMID24888375)) are in various stages of AOP development, ranging from a correlative AOP with hypothesized linkages to a strongly supported AOP with broad applicability across species and life stages. Despite the differing development stages, comparative use of the SCF indicates that the proposed application for each case study (e.g., "category formation" or "hazard assessment") is reasonable based on the strength of the supporting evidence for each. For the AOPs for which the submitter addressed application, this analysis demonstrates that the framework can be useful to inform appropriate applications based on the type and amount of data supporting an AOP.

Activities Aimed at Harmonizing the International Implementation of Test Methods or Approaches that Accomplish the 3Rs

Mei-Chun Lai[1], Aryenish Birdie[1], and Amy Clippinger[2]

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Test methods or approaches implementing the replacement, reduction, or refinement of animals (3Rs) have increased substantially over the past several years due to several factors, including the ethical concerns over the use of animals, the cost and time involved in traditional animal tests, and the potential for greater human relevance and predictive power of *in vitro* and *in silico* tests. To capitalize on progress in the 3Rs and encourage the use of new test methods and approaches in the regulation of products throughout the world and in their respective regions, International Cooperation on Alternative Test Methods (ICATM) and its member organizations were formed. However, there remains a troubling gap in the implementation of new approaches. Since member countries maintain their own national data requirements, as determined by legislative or regulatory statute, some test methods become duplicative. Some regions or member countries accept particular 3Rs approaches and some do not, giving a company wishing to market a product in multiple regions limited options for reducing animal testing. Here we present case studies demonstrating this lack of harmonization including requirements to conduct a 1-year dog toxicity study and *in vivo* skin irritation testing. Further, we propose that ICATM should take action to increase international harmonization in the acceptance of 3Rs test methods and approaches. As regulatory toxicology transitions away from the use of animals, and regulatory agencies, NGOs, and industry stakeholders devise and propose new and innovative ways to accomplish progress in the 3Rs, it becomes more important than ever for groups like ICATM to show leadership and encourage its governments to harmonize as much as possible with approaches other governments are taking to reduce and replace the use of animals for regulatory testing. We also suggest a number of potential activities that ICATM could pursue.

An In Silico Skin Absorption Model for Fragrance Materials

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Fragrance materials are widely used in cosmetics, fine fragrances, and other consumer products. At the Research Institute for Fragrance Materials (RIFM) we evaluate the safe use of fragrance ingredients. Dermal absorption is an important parameters in refining systemic exposure for topically applied fragrance materials. Currently, in RIFM's safety assessment process, a 100% dermal absorption default value is applied for materials without experimental data. However, as discussed by Kroes at al. in their 2007 publication, "the assumption of 100% absorption is not scientifically supportable" and proposed an *in silico* methodology to assign a conservative skin absorption value based on the material's maximum flux (Jmax). Jmax may be calculated by using QSAR models, that determine octanol/water partition coefficient (Kow), water solubility (S) and permeability coefficient (Kp). To apply Kroes et al. methodology specifically for fragrance materials, each of these QSAR models was carefully evaluated and refined resulting in a detailed model workflow we refer to as SAM (Skin Absorption Model). SAM was developed using 105 materials with fragrance specific physicochemical characteristics and experimental Kp values by updating Potts and Guy's proposed Kp model. SAM was validated by using a set of 133 materials with fragrance specific physicochemical characteristics that had experimentally determined skin absorption data using human or pig skin in either *in vitro* or *in vivo* methods. All resulted in predicted values fitting Kroes et al. three proposed skin absorption values based on Jmax ranges (see Figure 2). Our findings demonstrated that SAM may be conservatively applied when fragrance materials lack dermal absorption data.

Dual Assessment of Kinase Pathway Perturbation by Combining a Label-free Cell Based Assay with Global Kinase Activity Profiling.

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Environmental pollutants that impact critical biological pathways involved in human development and physiology can have unpredictable effects on health. Among these critical pathways, various receptor tyrosine kinases and serine-threonine kinases are known or suspected mediators of the effects of numerous environmental toxicants. The interconnected nature of these pathways, and their finely-tuned spatial and temporal regulation, make them simultaneously broad targets for environmental pollutants and challenging to efficiently model in the laboratory. We have performed a proof-of-concept study to evaluate the potential of combining two complementary technologies to accelerate identification of molecular and cell physiological changes predictive of toxic effects due to modulation of kinase pathways. Label-free cell-based assays provide an opportunity to assess the impact of toxicants on biological pathways in a relatively holistic and biologically relevant context compared to traditional assays, and can help identify key time points for deeper molecular analyses. Kinase activity profiling allows for global assessment of pathways perturbed due to modulation, and network mapping of the perturbed pathways can provide new insights into toxicity mechanisms. A431 human epdiermal carcinoma cells are known to express high levels of the epidermal growth factor receptor (EGFR) tyrosine kinase and to exhibit activation of EGFR and downstream pathways. Treatment with known EGFR modulators, including the natural ligand (EGF) and a synthetic inhibitor (Erlotinib) resulted in changes in cell morphology and attachment detected in the label-free assay. At key time points defined by this assay, cell lysates were collected and analyzed in the kinase activity profiling assay. Network analysis revealed that known EGFR substrates and downstream pathway members were activated or repressed as expected in response to the modulator treatments. In addition, novel pathway perturbations were observed in the activity assay, including pathways linking back to the observed morphology and attachment changes observed in the live cell assay.

Molecular and Cellular Effects of Rotenone and MPP+ in a 3D Human Dopaminergic Neuronal Model In Vitro

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The limitations of current Parkinson Disease (PD) model and the consequent poor advances in novel therapies have raised increasing concerns in recent years. For this reason, more efforts must focus on the characterization and development of physiologically relevant human cell models, which can predict the contributions of chemicals to PD. Rotenone and MPP+ exposure have been associated with PD in patients. There are several cell lines, which are usually used to study PD, but they have several limitations, not human origin (rat PC12 cells, or primary cultures), or cancer cells (neuroblastoma cell line, SH-SY5Y), all existing cellular PD models are traditional monolayer cultures. With this in mind, we have recently created a 3D dopaminergic model using LUHMES progenitor cell line, which is differentiated over 15 days (expressing TH, NeuN, β-TubIII and SYN1) and can be kept in culture for up to 21 days (twice longer than 2D cultures of the same cells). Using this model, cell viability, mitochondria dysfunction, neurite outgrowth and arborization, and perturbations in gene expression were measured after short-term (24 and 48h) exposures to rotenone and MPP+. Cell viability studies showed 3D cultures were less sensitive to these compounds than 2D cultures. Furthermore, we were able to see changes in expression of energy metabolism and stress response genes (ATF4, ASS1, CBS, CTH, MLF1IP, SHMT and TYMS) at non-cytotoxic concentrations (0.1μM rotenone, 10μM MPP+) as well as a dose-dependent decrease in mitochondria functionality using MitoTracker®. We visualized toxicant-induced changes in neuronal morphology by confocal microscopy of co-cultures of wild type and RFP-expressing LUHMES. In the next steps we will study short term exposures (24, 48 h) with a recovery period (14 days) and measure the effects on differentiation (gene expression), viability (LDH, MitoTracker, and cellular ROS) and morphology (confocal imaging). Our 3D model mimics *in vivo* physiology more closely than existing 2D *in vitro* models and therefore promises to identify molecular and cellular effects of neurotoxic compounds on dopaminergic neurons. This novel tool could strengthen the reliability of *in vitro* methods for PD risk assessment.

Replacement for the Draize Eye Irritation Test Using Human Donor Corneoscleral Explants Not Used for Transplantation

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Identification of the correct endpoints that drive the classification of test substances in the Draize eye irritation test is of critical importance in the development of its *in vitro* replacement. By taking corneal endothelial damage as the most important end point and application of an easy and inexpensive technique to quantify this damage, we have developed the first *in vitro* method that full fills the requirement of regulatory agencies: a single method capable of identifying test substances in all four categories of eye irritation (severe/corrosive [75-96% damage], moderate [14-36% damage], mild [4-10% damage], and non-irritant [<1.4% damage]). A totally animal- and serum-free system for chemical toxicity testing predicted with 100% accuracy the level of eye irritancy of 17 substances covering the full range of eye irritancy, including two severe/corrosive ones missed by bovine corneal opacity and permeability (BCOP) and isolated chicken eye (ICE) tests. Used as control vs test substance-treated (at 1:10 dilution for liquids and 2% [w/v] for solids), paired human donor corneoscleral explants unsuitable for transplantation were incubated in a serum-free medium (90 min, 37°C) under 95%/5% air/CO_2, followed by staining of the corneal endothelium with alizarin red S, which stained areas of damage in the corneal endothelium. Panoramic view of the whole corneal endothelium was analyzed using the Photoshop computer software to quantify alizarin red-stained areas. Severe/corrosive irritants (4% sodium hydroxide, chlorohexidine, 15% sodium lauryl sulfate, sodium oxalate, imidazole, 30% trichloroacetic acid) caused pan-corneal endothelial adhesion of alizarin red S and showing more than 75% corneal endothelial damage. Moderate (2,6-dichlorobenzoyl chloride, ethanol, acetone, ammonium nitrate) and mild (1% sodium hydroxide, 0.4% sodium hydroxide) irritants damaged the corneal endothelium at discrete areas resulting in quantifiable alizarin red S staining of 36-14%, and 10-4% of the corneal endothelium, respectively. Five eye lubricant drops sold over the counter [soothe, GenTeal A, Refresh, Blink, GenTeal B]) caused no (<1.4%) damage.