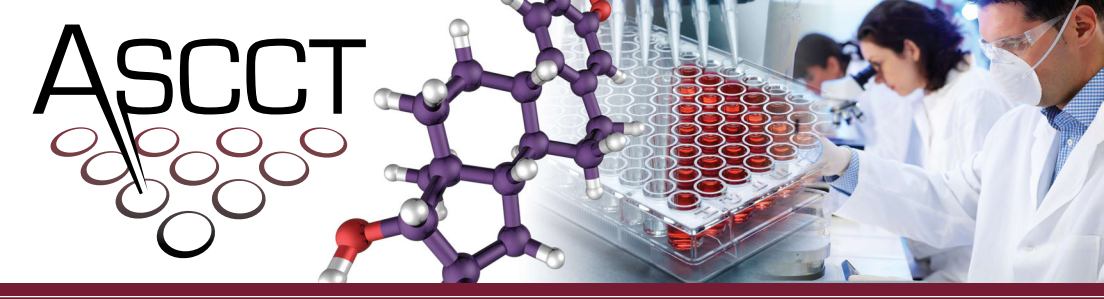

2nd Annual Meeting of the American Society for Cellular & Computational Toxicology

October 31, 2013
Lister Hill Auditorium, NLM, NIH
Bethesda, MD

President's Welcome

On behalf of the entire Board of Directors (Marilyn Aardema, Rodger Curren, Jack Fowle, Marianna Gaça, Thomas Hartung, Erin Hill, and Kristie Sullivan) of the American Society for Cellular and Computational Toxicology (ASCCT), I'd like to welcome everyone to our second annual meeting. We have a very exciting program planned for you today, and we hope that all of you will enjoy the presentations and take time to interact with the speakers and your fellow ASCCT members. Thankfully the Government shutdown has ended in time for us to enjoy the hospitality of Lister Hill Auditorium!

2013 has been filled with a number of events important to ASCCT members. One of the more notable, of course, was the imposition in Europe of the second stage of a marketing ban for cosmetics containing animal tested ingredients. Although the use of ingredients which had been subjected to acute toxicity tests had been banned since 2009, the current incarnation has extended the ban to repeat dose studies, e.g. skin sensitization, reproductive toxicity studies, systemic toxicity, etc. These requirements have spawned a number of new programs in which cellular and computational scientists will play a major role. …and it's likely that the opportunities for ASCCT members to provide their important scientific input will be available for quite a few years to come. Clearly the value of an American Society for Cellular and Computational Toxicology is obvious!

Today's program highlighting "Practical Applications of Emerging Scientific Tools" should have a little bit of something for everyone, be they cellular focused or computational focused. We lead off with Dr. Donald Ingber of the Wyss Institute presenting their program of "human on a chip". This concept of utilizing human mini-organs interconnected by an artificial vascular system has been the vision for many of us for the solution to the challenges possessed by replacing animal-based repeat dose systemic toxicity. Next will be Dr. Thomas Knudsen of the US EPA with an update on "Virtual Embryological Systems". In contrast to human on a chip, we'll be exposed to an embryo created by software. Hopefully this will stimulate some exciting discussions with our computational-based members.

These two talks will then be followed by a panel discussion addressing how these new tools, and others like them, can actually be put to use in risk assessments of new chemicals and formulated products.

The afternoon will feature four oral presentations from ASCCT members chosen from the submitted abstracts. Supporting these talks will be our membership poster session. Please spend some time visiting the posters and interacting with your fellow ASCCT members.

I want to thank each one of you for becoming an ASCCT member, and in many cases also convincing your company management to contribute to the society. There are now nearly 150 individual members of the society, and we have 13 institutional sponsors.

Finally – just as I did last year - I want to urge all of you to take the opportunity at this meeting to introduce yourself to someone new and initiate the scientific networking that is so important to each of our professional successes. The ASCCT was envisioned as a platform where scientists from the computational and cellular sides of toxicology could freely exchange ideas. Please do it!!

Your president,
Dr. Rodger Curren

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Meeting Sponsors

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The Future is Here:
Practical Applications
of Emerging Scientific Tools

2nd Annual Meeting of the
American Society for Cellular and Computational Toxicology

October 31, 2013
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8:30 AM	Welcome *Rodger Curren, ASCCT President*
8:45 AM	Human Organs on Chips as Replacements for Animal Testing *Donald E. Ingber, Wyss Institute*
9:45 AM	Virtual Embryological Systems: Challenges for Predictive Toxicology *Thomas Knudsen, US Environmental Protection Agency*
10:45 AM	Panel Discussion: Practical Applicability of New Tools *Moderator: John "Jack" Fowle, Science to Inform* *Panelists: Suzanne Fitzpatrick, US FDA* *Steven Bradbury, US EPA* *Amy Clippinger, PETA* *Edward Carney, The Dow Chemical Company*
11:30 AM	Lunch, Poster viewing
1:30 PM	Cultured Porcine Cornea Assay Using Confocal Microscopy for High Resolution Detection and Quantification of Sub-Mild Ocular Irritation *Michelle Piehl, MB Research Laboratories*
1:55 PM	Identification of Pathways of Developmental Neurotoxicity for High Throughput Testing by Metabolomics *Helena Hogberg, CAAT, Johns Hopkins University*
2:20 PM	Human Multi-cell Type 3D Liver Microtissues for Hepatotoxicity Testing *Jens Kelm, InSphero, Inc.*
2:45 PM	RIFM's Framework for In Silico Evaluation of Fragrance Materials *Sneha Bhatia, Research Institute for Fragrance Materials, Inc.*
3:30 PM	Break, Poster viewing
3:45 PM	ASCCT Business Meeting *Outline of Activities & Finances* *Election of Board Members*
4:30 PM	Cocktail Reception, addt'l poster viewing
5:30 PM	Close of Meeting

Poster Abstracts

Using Weighted Correlation Network Analysis Algorithms to Infer Networks from Metabolomics Data

Alexandra Maertens, Vanessa Sa-Rocha, Thomas Luechtefeld, Mounir Bouhifd, Thomas Hartung, Andre Kleensang

Johns Hopkins Bloomberg School of Public Health, Centre for Alternatives to Animal Testing, Baltimore MD, USA

Metabolomics, as the high-content -omics technology closest to the phenotype, will likely become an increasingly important aspect of systems toxicology alongside transcriptomics. While metabolomics produces many of the same challenges as other high-content methods - namely, how to integrate the surfeit of data into a meaningful framework - but at the same time, it has some unique challenges. In particular, metabolomics lacks the large-scale, integrated databases that have been crucial to the analysis of transcriptomic and proteomic data. Although a more-or-less complete "parts list" and wiring diagrams exist for transcriptomic and proteomic networks, establishing the identity of a putative metabolite and understanding the metabolites' biological role are more difficult in metabolomics than for other high-content technologies. Correlation analysis of microarray data is a fairly common approach for inferring connections among genes, and can be used for metabolomics data as well – although the biological significance of correlation is more ambiguous in metabolomics compared to transcriptomics.

In the NIH Human Toxome project, we used a dataset derived from MCF-7 cells treated with five doses of estrogen at five different time points. Using WGCNA[1] – which establishes a weighted network as opposed to a simple Boolean network - we derived a network that clustered the metabolites into biologically relevant modules, and compare the advantages of this approach compared to other methodologies that are dependent on accurate annotations, such as over-representation analysis of fold-changes in metabolites and quantitative enrichment analysis.

[1] Langfelder, H. et al. "WGCNA: an R package for weighted correlation network analysis." BMC Bioinformatics 9.1: 559 (2008).

Predicting Ocular Irritation of Surfactants Using the Bovine Corneal Opacity and Permeability

Jackie E. Bader, Kimberly G. Norman, Hans Raabe

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

The Bovine Corneal Opacity and Permeability (BCOP) assay is an ex vivo test used to evaluate ocular irritation. According to the OECD Test Guideline (TG) 437, the BCOP assay can be used to identify chemicals which induce severe/corrosive eye irritation and those that do not require classification. However, BCOP has historically under-predicted certain anionic surfactants, when tested according to the standard liquid protocol. TG 437 specifies that liquid and solid surfactants may be tested as 10% aqueous dilutions for 10 minutes (although alternate dilutions and exposure times may be conducted with scientific rationale). The relevant guidance document (GD) No. 160 suggests that solid and concentrated liquid surfactants may be diluted to 10% for testing. However, GD No. 160 further directs that surfactant-based formulations are usually tested neat, but could be diluted with justification, imparting some confusion in identifying the most appropriate test methods. Additionally, as part of the EPA classification of ocular irritation, the BCOP assay may be used to assess anti-microbial products with cleaning claims. Such products may contain surfactants and are generally tested neat for classification purposes. Since neither the basis for selecting the appropriate surfactant test methods, nor the justification for modifications are clearly presented in TG 437 or GD No. 160, we present on the testing of a few common surfactant ingredients, including sodium lauryl sulfate (SLS), Triton X-100, and benzalkonium chloride, and surfactant based formulations in the BCOP assay using standard and modified dilutions and exposures to elucidate the impact of these variables on eye irritation prediction. For example, in vitro scores of 20.7, 28.4, and 28.3 were obtained when testing SLS at concentrations of 50, 20, and 10% for 10 minutes, showing that irritation responses were not fully concentration-dependent. As a complement to the BCOP assay, histopathology was performed to assess the surfactant-induced corneal changes. Based upon these results, a framework for testing surfactant ingredients and surfactant-based formulations is proposed.

RIFM's Framework for In Silico Evaluation of Fragrance Materials

Sneha Bhatia, Lambros Kromidas, Jie Shen

Research Institute for Fragrance Materials Inc, Woodcliff Lake, NJ, USA

Fragrance materials are used widely in cosmetics, household products, and various other consumer products, hence it is crucial to assess the safety of these materials. The shift towards 3Rs and legislations such as REACH encourage the use of in silico tools for risk assessment. The pharmaceutical industry uses in silico tools for predictive safety assessment of new compounds before they enter the pipeline. Currently, the mainstream in silico models largely contain data on pharmaceuticals, hence using these models for non-pharmaceuticals such as fragrance materials, requires extensive filtration of the results and expert judgment. As such a procedure for conducting in silico assessments on fragrance materials using quantitative and qualitative structure activity relationships (Q)SARs, metabolism, and mechanistic domain is described. Strategies that can be incorporated to prioritize and reduce testing are also described.

The Use of a Quantitative Risk Assessment Tool for the Prioritization of Cigarette Smoke Toxicants for Reduction Research

D. Breheny, F. Cunningham, S. Fiebelkorn, D. Dillon and C. Meredith

British American Tobacco, Group R&D, Southampton, SO15 8TL, UK

The US Food and Drug Administration (FDA) have outlined seven research priority areas relating to tobacco products. Research area three focused on "reducing toxicity and carcinogenicity of tobacco products and smoke". This has been coupled with an increased interest in characterising individual tobacco smoke toxicants from the perspective of regulatory frameworks and tobacco product development focused on selective toxicant reduction.

We previously described the Margin of Exposure (MOE) model as part of a quantitative risk assessment paradigm for individual tobacco smoke toxicants. Computed MOEs enable segregation of toxicants into high and low priority groupings for risk reduction research depending on their relationship to the critical MOE value of 10,000. Here we propose further segregation of tobacco smoke toxicants into bandings based on their MOEs as follows: 1–10 (top priority), 10–100 (very high priority), 100–1000 (high priority), 1000–10,000 (medium priority), 10,000–1,000,000 (low priority), >1,000,000 (very low priority).

We applied this approach to the WHO Study Group on Tobacco Product Regulation (TobReg) list of 18 toxicants for mandatory lowering and monitoring, as well as the FDA abbreviated list of Harmful and Potentially Harmful Constituents (HPHC). In all, 23 toxicants were evaluated. The following toxicant classifications were obtained:

Top priority – Acrolein
Very high priority – Cadmium, Formaldehyde, Acrylonitrile
High priority – 1,3-butadiene, Acetaldehyde, Isoprene
Medium priority – Benzene, Toluene
Low priority – Benzo(a)pyrene

Ranking is based on the majority view for each toxicant. However, it is not always appropriate to apply these bandings. For 4-N-Nitrosomethylamino-1-(3-pyridyl)-1-butanone (NNK) and N-Nitrosonornicotine (NNN) typical MOE values lie both above and below 10,000, and for 2-aminonaphthalene there is only one MOE available. There were insufficient data to calculate MOE values for 10 remaining chemicals from the two lists. While additional experimental data are needed, this tool provides valuable information for prioritization of toxicants for risk assessment purposes.

Waterfall Microfluidics Culture Plate: A New Biodevice for Complex Cell Culture Systems

Maureen Bunger, Gabrielle Resh, Sam Tetlow, Randall McClelland

SciKon Innovation, Inc., Research Triangle Park, NC, USA

Increasingly, researchers are turning to more complex cell culture systems as a way to improve functionality and physiological relevance of in vitro cell culture experimental results. Ideally, such systems would allow researchers to build physiological model systems in a way that is compatible with existing lab equipment, accessible to common experimental endpoints, and with the versatility to adapt to multiple experimental designs. SciKon Innovations has developed an in vitro cell culture biodevice that consists of wells linked together through microfluidic channels and a syphon-drive system that controls flow through and between wells. The system allows for a "tempered" flow of chemical concentrations through wells either of a single lineage or between multiple lineages. The device advances nutrient transport through time-resolved gradients and was designed to retain traditional culture configurations. This affords users the option to evaluate networked –cell, -tissue, and -environment relationships. Here we describe the engineering and fluidics properties of the device and discuss potential applications for dynamic and kinetic dose-response experiments in support of cell- and pathway-based toxicology.

The Replacement Ocular Battery (ROBatt): An Integrated Testing Strategy for Ocular Irritation Classification

DR Cerven, D Hall, M Piehl, MR Carathers, GL DeGeorge

MB Research Laboratories, Spinnerstown, PA, USA

Alternatives to the Draize Rabbit Eye Test have been available since the1980s but none have yet been fully successful due to no appreciable regulatory adoption to date. To address this need, we have developed an integrated testing strategy (ITS) for ocular toxicity testing: the Replacement Ocular Battery (ROBatt).

ROBatt incorporates four alternative ocular irritation assays – the Chorioallantoic Membrane Vascular Assay (CAMVA), the Bovine Cornea Opacity/Permeability test (BCOP), the Porcine Cornea Opacity/Reversibility Assay (PorCORA), and the Porcine Confocal Assay (PorFocal) into a logical testing approach for ocular irritancy potential ranging from non-irritant to ocular corrosion. A decision tree was devised that integrated these assay and allows for a thorough evaluation and categorization of test materials. Fifty two chemicals were selected from the ECETOC Technical Report No. 48 - Eye Irritation: Reference Chemicals Data Bank (Second Edition). The ECETOC data and classifications were supplemented by data provided by the FDA and EPA. These 52 chemicals were tested using the ROBatt ITS to establish criteria that would lead to regulatory classification of chemicals with respect to ocular toxicity. The results are reported here and compared with in vivo observations to provide the basis to evaluate the performance of ROBatt as an informative and efficient tiered testing strategy to categorize chemicals into regulatory classification without using the Draize test or employing live animals.

Inter-Laboratory Validation of an In Vitro Method to Classify Skin Sensitizers

Amy J. Clippinger[1], James M. McKim, Jr[2], Donald Keller[2], Paul C. Wilga[2], Hilda Witters[3] and An R. Van Rompay[3]

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[2]CeeTox Inc., Kalamazoo, MI, USA
[3]Flemish Institute for Technological Research, Mol, Belgium

Allergic contact dermatitis presents a concern for developers of personal care, chemical, pharmaceutical, and medical device products. The development of non-animal methods to assess skin sensitization is a priority due to the EU cosmetics testing ban, the 2018 REACH deadline, and the goal of reducing animal use.

This study builds upon previous studies (McKim et al, 2010; McKim et al, 2012) showing that CeeTox's in vitro SenCeeTox® assay can correctly identify and categorize chemical sensitizers when used in-house. The aim of this project was to further validate the SenCeeTox® assay by conducting an inter-laboratory validation at the Flemish Institute for Technological Research (VITO).

In this study, MatTek's three-dimensional human skin model, EpiDerm (EPI-296, EpiDerm 96-well reconstituted human epidermis), was treated in triplicate with six concentrations of each test article. Test articles were run in a blinded manner. The test articles evaluated were: metol, isoeugenol, 2,3-butanedione, 2-mercaptobenzothiozol, eugenole, 1-chloro-2,4-dinitrobenzene, glycerol, 2-hydroxyethylmethacrylate, 2-hydroxyethylacrylate, and lactic acid. Following 24 hr exposure to the test articles, the following endpoints were measured: 1) cytotoxicity, 2) the ability of each chemical to directly react with glutathione, and 3) expression of key genes. The gene expression levels of seven target genes controlled by the Nrf2/Keap1/ARE signaling pathway were examined: NADPH-quinone oxidoreductase 1, aldoketoreductase 1C2, interleukin 8, cytochrome P450 1A1, aldehyde dehydrogenase 3A1, heme-oxygenase 1, and glutamate cysteine ligase catalytic subunit C. The data were then analyzed in a blinded manner using a proprietary algorithm to predict each chemical's likelihood of causing a human sensitization reaction.

The results confirm the inter-laboratory reproducibility of SenCeeTox® which accurately predicted the ability to elicit a sensitization reaction for all 10 blinded compounds tested at VITO. Furthermore, it correctly predicted the sensitization potency category for 9 out of the 10 compounds, missing the 10th compound by only one potency category. In conclusion, SenCeeTox® can predict the sensitization potency of chemicals ranging from non-sensitizers to strong-extreme sensitizers. Because results from the SenCeeTox® assay have been so promising, further validation of this assay will be undertaken by Cosmetics Europe, after which all results will be submitted to EURL ECVAM.

A Dermal Sensitization Assay Using SkinEthic™ RHE

GL DeGeorge and M. Troese

MB Research Laboratories, Spinnerstown, PA, USA

International regulatory agencies, as well as animal welfare groups, are seeking in vitro assays for assessing the toxicity of chemicals and products. One of the most difficult challenges has been to develop non-animal tests for skin sensitization. These challenges include the difficulty in replicating key pathways in the mechanism of chemical allergy, the proprietary nature of some test candidates being considered by ECVAM and other agencies, and the high cost of most of these test methodologies. As an outcome of the Sens-it-iv project in Europe, an Interleukin-18 (IL-18) response assay in monolayer keratinocytes to detect sensitizers was developed by Corsini and colleagues in 2009. Here we report enhanced release of IL-18 into the culture medium by SkinEthic™ RHE treated with dermal sensitizers, but not with irritants or non-sensitizers. Also, sensitizer-induced IL-18 release by RHE tissues was observed to occur in a concentration-dependent manner. RHE tissues were exposed to test substances for 24 hours. Data were expressed as a Stimulation Index (SI) calculation, which was the ratio of chemical-treated tissues indexed to vehicle-control tissues. An SI ratio of >2.0 was considered a positive result for a dermal sensitizer. A range of known sensitizers from slight to severe was tested, along with nonsensitizers and irritants. IL-18 was measured by an ELISA kit and tissue viability was measured using the MTT conversion assay. Of the twelve known positive sensitizers tested, ten were correctly predicted. In addition, six of seven irritants and nonsensitizers were correctly predicted. Overall accuracy of the assay was calculated to be 84%.

In conclusion, an in vitro assay for dermal sensitizers was demonstrated in SkinEthic™ RHE, using IL-18 as an endpoint. This assay is promising for identification of sensitizers with high accuracy and predictivity.

Evaluation of the Eye Stinging Potential of Baby Shampoos by Assessing TRPV1 Channel Activity

Anna Forsby[1], Kimberly Norman[2], Lindsay Krawiec[2], Johanna EL Andaloussi-Lilja[1], Jessica Lundqvist[1], Beata Wojcik[3], Vincent Walczak[3], Rodger Curren[2], Katharine Martin[3] and Neena Tierney[3]

[1] Department of Neurochemistry, the Arrhenius Laboratories for Natural Science, Stockholm University, Stockholm, Sweden
[2] Institute for In Vitro Sciences, Inc., Gaithersburg, MD, USA
[3] Johnson & Johnson Consumer and Personal Products Worldwide, Skillman, NJ, USA

The Transient Receptor Potential Vanilloid type 1 (TRPV1) receptor is one of the most well characterized pain-inducing receptors. In this study we sought to predict eye stinging of 19 baby bath and shampoo formulations by studying TRPV1 activity. We employed the NociOcular test, a novel recombinant neuronal in vitro model with high expression of functional TRPV1 channels, to test shampoo formulations containing surfactants, preservatives, and fragrances (sodium laureth sulfate, cocoamidopropylbetaine, cocoglucoside, sodium benzoate, quaternium-15, etc.). The increase in intracellular free Ca2+ was analyzed by fluorescence during exposure. TRPV1-specific Ca2+ influx was abolished when the TRPV1 channel antagonist capsazepine was applied to the cells prior to shampoo samples. The positive control (an adult shampoo), was the most active sample tested in the NociOcular test. The negative control (marketed baby shampoo) was negative in both tests. Seven of the formulations induced stinging in the human test, and of those six were positive in the NociOcular test. Twelve of the formulations were classified as non-stinging in the human test, and of those ten were negative in the NociOcular test. None of the established in vitro tests for eye irritation were able to correctly predict the human stinging sensation of the baby products. Our data support that the TRPV1 channel is a principle mediator of eye stinging sensation induced by baby bath and shampoo formulations and that the NociOcular test may be a valuable in vitro tool to predict human eye stinging sensation.

Characterization of a 3-Dimensional Dopaminergic Cell Model for Developmental Neurotoxicity Studies

Georgina Harris, Rober Bachinski, Helena T Hogberg, David Pamies, Thomas Hartung, Lena Smirnova.

Center for Alternatives to Animal Testing (CAAT), Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

The increasing number of neurodevelopmental disorders, a growing concern about adverse effects of possible neurotoxicants on developing organisms and, the low prediction of current in vivo tests, have led to the need for research focused on in vitro methods for developmental neurotoxicity. As an in vitro cell model we are using Lund human mesencephalic (LUHMES) cells derived from a female human fetal (8 weeks) brain, which can be fast and homogenously differentiated into post-mitotitc dopaminergic neurons.

In our work we have adapted and modified the LUHMES differentiation protocol for monolayer (2D) toxicant exposure and in addition, characterized a 3D human neuronal model using constant gyratory shaking as used for the 3D rat primary aggregating brain cell model. Monolayer LUHMES cells grown in differentiation medium could be kept no longer than 9 days while floating aggregates could remain in culture for over 21 days. Two different differentiation protocols for 3D cultivation (with and without pre-differentiation step) were compared and characterized by RT-PCR with neuronal marker genes and miRNAs. Furthermore, differentiation efficiency in 2D and aggregate cultures was studied by immunocytochemistry and flow cytometry with a neuronal marker; cell viability was analyzed using annexin V/7-AAD staining. Size of differentiated aggregates was monitored up to day 21 of differentiation. We observed increase of NeuN positive cells (mature neurons) and decrease Ki-67 positive cells (proliferating cells) in the course of differentiation by flow cytometry. We observed induction in gene expression of the neuronal markers (β-III-Tubulin, synapsin I, DAT and TH) as well as neural-specific/enriched miRNAs (mir-124, mir-9, mir-132, mir-133b, mir-137, mir-128) during neuronal differentiation in both 2D and 3D cultures. Aggregate size increased over time which was reduced by pre-differentiating the cells in monolayer culture for two days before cultivating as aggregates. Immunocytochemistry data showed that during early stages of differentiation, 3D cultures did not contain apoptotic cells. However, 15-30% of the cells within aggregates were apoptotic (Annexin V positive) or necrotic (AnnexinV and 7-AAD positive) at later time points (9-21 days). We are currently further optimizing this protocol to obtain purely differentiated neurons within 3D aggregates for the study of developmental neurotoxicity.

Identification of Pathways of Developmental Neurotoxicity for High Throughput Testing by Metabolomics

HT Hogberg, R Bachinski, M Bouhifd, G Harris, A Kleensang, S Odwin-DaCosta, D Pamies, L Smirnova, L Zhao, and T Hartung

The Johns Hopkins University, Bloomberg School of Public Health, Center for Alternative to Animal Testing, Baltimore, MD, USA

The National Research Council report from 2007 "Toxicity Testing in the 21st Century: A vision and a strategy" has created an atmosphere of departure in the US. It suggests moving away from traditional (animal) testing to modern technologies based on pathways of toxicity. An area of toxicology where Tox-21c could have a significant impact is developmental neurotoxicity (DNT). There is concern that exposures to environmental chemicals contribute to the increasing incidence of neurodevelopmental disorders in children. However, due to lack of DNT studies only very few substances have been identified as developmental neurotoxicants. Current animal test strategies for DNT have several limitations: high costs ($1.4 million per substance) as well as time consumption. In addition, there are scientific concerns regarding the relevance of these studies for human health effects. Moving towards a mechanistic science can help us identify the perturbed pathways that likely lead to these adverse effects. DNTox-21c is a CAAT project funded by the FDA that aims to find pathways of developmental neurotoxicity using a metabolomics approach.

A 3D rat primary neuronal organotypic model was exposed from day 7 up to 21 to suspected (developmental) neurotoxicants including pesticides (carbaryl, chlorpyrifos, lindane maneb), drugs (fluconazole, isotretionin, phenytoin, terbutaline, valproic acid) and metals (CaCl2, PbCl2). Mass spectrometry based metabolomics measurements were performed and quantitative measurement of genes expressed in different cell types (neural precursor cells, neurons and glial cells). Treatment with the different compounds significantly modified the expression of selected genes, related to the different stages of neuronal and/or glial cell development and maturation. Moreover, the mass spectrometry analysis showed differences in metabolite levels between control and treated cells.

Signatures of changed features have been putatively identified and associated to possible perturbed pathways. E.g. metabolites involved in the biochemical pathway of the neuronal specific metabolite N-acetyl aspartate (NAA) (malate, aspartate, NAA and glutamate) were significantly decreased after exposure to lead chloride and maneb. In patients, the level of NAA has shown to be decreased in numerous neuropathological conditions such as brain injury, stroke and Alzheimers. It also indicates neuronal/axonal loss or compromised neuronal metabolism. Obtained data suggests that metabolomics could be a promising tool for developmental neurotoxicity testing.

Funded by the FDA.

Human Multi-Cell Type 3D Liver Microtissues for Hepatotoxicity Testing

Jens Kelm, Stewart Hunt

InSphero, Inc, USA

Current 2-dimensional hepatic model systems often fail to predict chemical-induced hepatotoxicity due to the loss of a hepatocyte-specific phenotype in culture. For more predictive in vitro models, hepatocytes have to be maintained in a 3-dimensional environment that allows for polarization and cell-cell contacts. Preferably, the model will reflect an in vivo-like multi-cell type environment necessary for liver-like responses.

Here we report the characterization of a multi-cell type microtissue model, generated from primary human hepatocytes and liver-derived non-parenchymal cells. The liver microtissues were generated by co-culture of liver cells in GravityPLUSTM hanging-drop plate. After tissue formation, the liver microtissues were transferred into GravityTRAPTM plates and cultivated further under serum-free conditions. Liver microtissues were stable and functional for 5 weeks in culture. Morphological characterization revealed presence of tight cell-cell contacts and glycogen storage. In addition, non-parenchymal cells (Kupffer cells, endothelial cells) were identified and shown to be distributed within the microtissue. Long culture life-time enabled long term toxicity testing of Acetominophen, Diclofenac and Troglitazone. In addition, Kupffer-macrophages were responsive to inflammatory stimuli such as LPS, which demonstrates the possibility to detect inflammation-mediated toxicity.

Herewith, we present a novel 3D liver model for routine testing in 96-well format capable of reducing the risk of unwanted toxic effects in the clinic.

The Human Toxicology Project Consortium: Advancing a New Paradigm for Assessing Chemical Safety

Marilyn Matevia

The Humane Society of the United States, Gaithersburg, MD, USA

The Human Toxicology Project Consortium (HTPC)* is a multi-stakeholder coalition working to accelerate the global implementation of a new paradigm in toxicity testing. The new paradigm is grounded in a mechanistic understanding of chemical-biological interactions, which allows a more predictive approach to risk assessment, and promises to generate better data more efficiently on the potential risks of chemicals to humans and the environment, all while moving away from a reliance on animal testing. To advance this paradigm, HTPC focuses on three core activities: (1) implementing the science, (2) communicating the science, to build understanding and consensus among stakeholders, and (3) lobbying/advocating for financial support and regulatory acceptance. Implementing the science involves identifying gaps in knowledge that will benefit from additional support and/or focus, and providing this support. To communicate the science to stakeholders (e.g., regulatory agencies, public health officials and providers, consumers, and advocates for environmental and animal protection), the HTPC is developing an educational series of web-based tutorials and articles. To speed the adoption and acceptance of new paradigm technologies, the HTPC works – in the US and internationally - to effect policy changes and cultivate research funding opportunities. The HTPC's impact can be measured in the following activities and achievements: organized, sponsored, and co-sponsored workshops and presentations at conferences/advisory panels; the launch of a web site that both reviews and promotes HTPC activities and explains the science to interested stakeholders; secured financial support from the US government for the necessary assessment methods and prediction tools in various US programs (e.g. the Tox21 partnership between EPA, FDA and NIH); and educational lobbying for pathway-based science as an underpinning of EUs Horizon 2020 programs.

*HTPC members and supporters include the Center for Alternatives to Animal Testing (CAAT), Dow, DuPont, ExxonMobil, the Hamner Institutes for Health Sciences, the Health and Environmental Sciences Institute (HESI), the Humane Society of the United States, Humane Society International, the Humane Society Legislative Fund, Johnson & Johnson, L'Oreal, Procter & Gamble, Toxicological Excellence for Risk Assessment (TERA) and Unilever.

3D In Vitro Brain Model for the Study of Neurotoxicity and Developmental Neurotoxicity

David Pamies[1], Joseph Bressler[1], Kimberly M Christian[3], Zhexing Wen[3], Georgina Harris[1], Georgia Makri[3], Cliona O'Driscoll[2], Bachinski Rober[1], Lena Smirnova[1], Thomas Hartung[1], and Helena T Hogberg[1]

[1] Centers for Alternatives to Animal Testing (CAAT) at Johns Hopkins and Konstanz, University, Germany Johns Hopkins University, Bloomberg School of Public Health, Baltimore, MD, USA
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[3] Institute for Cell Engineering, Department of Neurology, Johns Hopkins University, School of Medicine, Baltimore, MD, USA

Neuronal disorders have become one of the major public health challenges. The World Health Organization (WHO) has expressed a big concern about of the increase of neurological disorder follow by scarce service and resource, and the unprepared policy-makers. We have a limited understanding of the function of the central nervous system (CNS) and the complexity of the brain and neural circuits, especially during development and neuronal plasticity. There is a concern that chemical exposure might contribute to neurological disorders. However, the neurotoxic potential of many chemicals remain unknown as current in vivo (developmental) neurotoxicity tests are expensive and time consuming. Besides, the relevance of these tests for human effects is doubtful. Moreover, the difficulty of studying interactions between human genetics and environmental factors leads to lack of knowledge about the events that induce neurological diseases. Microphysiological systems (MPS) have been defined as cellular and organ microsystems with a multicellular architecture representing the characteristics and functions of human organs. The incorporation of these systems have put some light on the road, with the possibility to generate more complex in vitro human models that better simulate the organ biology and function. The recent discovery of induced pluripotent stem cells (iPSC) gives a range of possibilities allowing studies of individuals with different genetic backgrounds (e.g. human diseases models). For instance, iPSCs derived from patients have shown great potential for studying neurological diseases, such as Alzheimer, Down syndrome and Huntington's disease. Application of iPSCs combined with MPS gives the opportunity to better understand disease and can be a novel tool in different fields. Our group has established a 3D model consisting of aggregating cultures of iPSC derived neural cells. 3D aggregates have been positive to different neuronal markers staining after 4 weeks of differentiation. In addition, real-time PCR analyses show higher expression of various neuronal differentiation markers in 3D cultures compared to 2D culture (β-Tubullin III, Neurofilament-200, Tyrosine hydroxylase and Synapsin 1), showing a complex interaction between the different cell types. Here we present the application of this emerging technology and identify possible future directions of our developed 3D brain model from iPSCs.

Funded by NCATS grant "A 3D model of human brain development for studying gene/environment interactions" (1U18TR000547).

Cultured Porcine Cornea Assay Using Confocal Microscopy for High Resolution Detection and Quantification of Sub-Mild Ocular Irritation

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A critical need exists for a non-animal ocular irritation assay that is sensitive to sub-mild ocular irritation. We have developed a novel assay, PorFocal, which can quantify individual dead corneal epithelial cells in porcine corneas using confocal microscopy. PorFocal uses phosphate buffered saline (PBS) as a negative control and 0.01% benzalkonium chloride (BAK) as a positive control. In 17 experiments, 0.01% BAK always caused more cell death than PBS, and statistically ($p<0.05$) more in 15 of 17 replicates. The PorFocal assay detected a significant dose-response with BAK dilution series treatment. Treatment with 0.01% BAK-preserved lubricant eye drops showed a significant 3-fold increase in cell death versus the preservative-free version. To examine the potential of the PorFocal to detect human eye sting of a known stinging chemical (avobenzone), we compared a low avobenzone (LA) to a high avobenzone (HA) sunscreen. The HA caused significantly more cell damage (7-fold increase) than the LA. We compared PorFocal to an industry standard 3D reconstructed human tissue (RhT) ocular irritation assay. The RhT detected four of nine materials while the PorFocal assay detected eight of nine materials tested as statistically different ($p<0.05$) from PBS values. Overall, this indicates a great degree of sensitivity with PorFocal assay, heretofore not attainable by existing methods.

Combustible and Non-Combustible Tobacco Product Preparations Elicit Differential Cytotoxic and Genotoxic Responses in Cultured Cells

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Exposure to cigarette smoke or its constituent phases induces a range of cellular responses that include cytotoxicity, genotoxicity and inflammatory responses in several human cell culture models. The cellular effects of exposure to smokeless tobacco (ST), however, are less clear and appear to vary based on experimental conditions. To comparatively assess the effects of combustible (cigarette smoke) and non-combustible tobacco (ST), we prepared several tobacco product preparations (TPPs) and tested them in short-term cell culture. The TPPs included total particulate matter (TPM) and whole smoke conditioned medium (WS-CM) from 3R4F reference cigarettes, and extracts of 2S3 moist snuff in complete artificial saliva (ST/CAS). Oral cavity cells and peripheral blood mononuclear cells (PBMCs) were utilized to assess local and systemic effects, respectively. We used the nicotine content of the TPPs (termed equi-nicotine units) to compare the responses elicited by TPPs.

Treatment of cells with WS-CM and TPM, but not ST/CAS resulted in marked and dose-dependent cytotoxic responses as revealed by the EC50 values expressed in equi-nicotine units. WS-CM was generally more cytotoxic to all cell types, except normal human gingival epithelial cells (HGECs). EC50 values for WS-CM and TPM for HGECs and two oral cancer cell lines were lower compared to PBMCs, HL60 and THP1 cell lines, indicating cell-type differences. Although ST/CAS at higher doses caused measurable cytotoxicity, the EC50 values could not be achieved in these cell culture systems. Cytotoxic effects due to nicotine treatment were detectable only at higher concentrations, and the EC50 values were achieved at mM concentrations.

Exposure to TPM and WS-CM induced higher DNA damage, as measured by γ-H2AX levels (oral cavity cells and THP1 cells) and comet assays (oral cavity cells) relative to ST/CAS. Cell death induced by TPM was mediated by caspase 3- mediated apoptotic pathways, whereas WS-CM appeared to induce cell death through both apoptotic and non-apoptotic mechanisms. While nicotine (500µg/ml) induced DNA strand breaks were not detected in oral cells, nicotine caused a significant increase in strand breaks at 2000µg/ml in THP1 cells. Collectively, these findings show that TPPs differ in their cytotoxic and genotoxic effects in the following order: WS-CM>TPM>ST/CAS>>nicotine.

Pathway-based Approaches to Safety Assessment: Development and Use

Catherine Willett

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Pathway based approaches to the assessment of chemical toxicity are based on the understanding of the molecular interactions that occur – from the initial interaction with a living system (the molecular initiating event) - through a sequential progression of events, to the in vivo toxicity, or adverse outcome. This description, which has variously been described as "mode-of-action," "toxicity pathway" or "adverse outcome pathway" is a useful framework for organizing information and can assist in a number of toxicological assessments, including: informing chemical category formation and structure–activity relationships, increasing the certainty of interpretation of both existing and new information, and allowing the design of hypothesis-based assessment strategies. The applicability of a particular pathway to different aspects of hazard and risk assessment is proportional to the depth and certainty of information that supports it. For example, to use an AOP for building Quantitative Structure Activity Relationships (QSARs) of MIEs, there must be some solid evidence that the MIE is linked to the AO of interest, but the main focus of certainty would be the chemical and molecular characterization of the MIE itself. To use an AOP for hazard identification or prioritization of chemicals for further testing, strong evidence of the MIE-AO linkage is required, along with substantiation of one or more intermediate events.

Once a number of pathways have been described in sufficient detail, it will also be possible to use them to identifying key events for which tests can be developed; the tests would necessarily address a number of critical steps, thereby ensuring that all possible outcomes are adequately covered. As quantitative information is added to relationships between intermediate events, early events in an AOP can be used directly for risk assessment, without the need to assess the later steps pathway. At this stage, chemical assessment will be streamlined and toxicology transformed from a purely empirical to a predictive science. Case studies will be presented to highlight some of these uses as well as issues that remain to be resolved.

Speaker and Panelist Biographies

Steven P. Bradbury

Director, Office of Pesticide Programs
Office of Chemical Safety and Pollution Prevention
U.S. Environmental Protection Agency

Dr. Steven Bradbury was named Director of the Office of Pesticide Programs (OPP) in March 2010, where he is responsible for the overall leadership and management of the pesticide programs under the authority of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA); the Federal Food, Drug and Cosmetic Act (FFDCA); the Food Quality Protection Act of 1996 (FQPA); the Endangered Species Act; and, the Pesticide Registration Improvement Act (PRIA). Dr. Bradbury has management and operational responsibilities over EPA's largest Headquarters' program office, with approximately 700 employees and a budget of about $150 million. Dr. Bradbury previously served as OPP's Deputy Director for Programs. From 2003 to 2008, he was Director of OPP's Special Review and Reregistration Division and OPP's Environmental Fate and Effects Division, where he was responsible for development of national regulatory decisions for existing pesticides, pesticide drinking water exposure characterizations and ecological risk assessments. Dr. Bradbury joined EPA's Office of Research and Development in 1985, and from 1999 to 2002, Dr. Bradbury was the Director of the Mid-Continent Ecology Division. As a research scientist and senior executive in EPA, Dr. Bradbury has played a leadership role in advancing the development and implementation of „21st Century" toxicology techniques for human health and ecological risk assessments. Dr. Bradbury has a B.S. in Molecular Biology from the University of Wisconsin-Madison and M.S. and Ph.D. degrees in Toxicology and Entomology from Iowa State University.

Edward W. Carney

Scientific Director for Toxicology Research
Dow Chemical Company

Edward (Ed) Carney is Scientific Director for Toxicology Research at The Dow Chemical Company in Midland, Michigan, where he is responsible for overall science strategy, as well as leadership of Dow's new Predictive Safety Assessment program. He also is involved with numerous external organizations including US EPA's Chartered Scientific Advisory Board and Board of Scientific Counselors, ECVAM Scientific Advisory Committee, Hamner Institutes Board of Directors, Teratology Society (Past-President), University of Michigan School of Public Health (adjunct faculty), and University of Surrey (UK) Master's Programme in Toxicology (lecturer). He holds a PhD in Reproductive Physiology from Cornell and conducted postdoctoral research in molecular developmental biology at Mount Sinai Hospital in Toronto.

Amy Clippinger

Science Advisor
People for the Ethical Treatment of Animals (PETA)

Dr. Amy Clippinger is Science Advisor for People for the Ethical Treatment of Animals (PETA). Her work focuses on promoting the use and development of human-relevant non-animal test methods to meet regulatory requirements. She is a member of the Society of Toxicology, the ISO technical committee on nanotechnology, and participates on the OECD Working Party for Manufactured Nanomaterials as a part of ICAPO. She has a Ph.D. in Cellular and Molecular Biology and Genetics and, before coming to PETA, completed a post-doctoral fellowship in the Cancer Biology Department at the University of Pennsylvania.

Suzanne Fitzpatrick

Senior Science Policy Analyst
Office of Science and Health Coordination
US Food and Drug Administration

Dr. Suzanne Fitzpatrick is the Human Protection Administrator for the FDA Institutional Review Board. In this position, she drafted the Standard Operating Procedures for the FDA IRB and oversees its daily activities. She is also working on the oversight program for quality assurance of all FDA sponsored research. She is a member of the FDA Human Protection Steering Committee, the FDA Human Subjection Protection/Biomonitoring Steering Committee, and the 21 CFR 50.24 consultative review committee. Dr. Fitzpatrick is also a co-investigator on an FDA grant entitled "Pediatric Assent in Adolescent Research Participants" in collaboration with NCI/NIH and Walter Reed Army Medical Center. She is the FDA National Environmental Protection Act (NEPA) liaison to the Council for Environmental Quality at the White House. She represents FDA on several Office of Science and Technology Policy Committees (OSTP) including the CNER Subcommittee on Toxics and Risk, the OSTP Subcommittee on Health and the Environment, and the CNER Subcommittee on Endocrine Disruptors. She chairs, with EPA and USGS, the OSTP Interagency Working Group on Human and Veterinary Pharmaceutical in the Environment, whose charge is to leverage research strategies in this area across the different federal agencies. She is also a member of the Interagency Committee on Validation of Alternative Animal Models and a board certified toxicologist. She is the past president of the American College of Toxicology and also a past member of its board of councilors. Currently Dr, Fitzpatrick is the President Elect of the Nation's Capital Chapter of the Society of Toxicology (SOT), a Councilor for the Regulatory and Safety Specialty Section of and a member of the K-12 SOT Education Committee. She is also an adjunct professor at Johns Hopkins University, Zanvyl Krieger School of Arts and Science. Dr. Fitzpatrick received her BA from the University of California at San Diego and her PhD from Georgetown University.

John “Jack” Fowle

Principle
Science to Inform, LLC

Dr. John R. “Jack” Fowle III is the principal of Science to Inform, LLC, where he advises clients about the use of science to inform decisions regarding environmental risk and in the development and use of alternatives to animal testing. Before 2012 he was the Deputy Director of the U.S. Environmental Protection Agency’s (EPA) Health Effects Division in the Office of Pesticide Programs and prior to that he served as Director of EPA’s Neurotoxicology Division and Assistant Laboratory Director at the National Health and Environmental Effects Research Lab. During his tenure at the EPA, a large focus of his work was the development and implementation of in vitro and computational toxicology approaches to assess the health risks of chemicals and pesticides. Dr. Fowle currently serves on a number of boards and committees, including the Institute of In Vitro Sciences, the American Society for Cellular and Computational Toxicology, the Society of Toxicology’s In Vitro and Alternative Methods Specialty section, and the Evidence Based Toxicology Consortium at Johns Hopkins University. He received both his baccalaureate and doctoral degrees in genetics from George Washington University in Washington, DC.

Donald E. Ingber

Founding Director
Wyss Institute for Biologically Inspired Engineering

Donald E. Ingber, M.D.,Ph.D. is the Founding Director of the Wyss Institute for Biologically Inspired Engineering at Harvard University, the Judah Folkman Professor of Vascular Biology at Harvard Medical School and Boston Children's Hospital, and Professor of Bioengineering at the Harvard School of Engineering and Applied Sciences. Dr. Ingber is a founder of the emerging field of biologically inspired engineering, and at the Wyss Institute, he leads the Biomimetic Microsystems platform in which microfabrication techniques from the computer industry are used to build functional circuits with living cells as components. His most recent innovation is a technology for building tiny, complex, three-dimensional models of living human organs, or "organs on chips", that mimic complicated human functions as a way to replace traditional animal-based methods for testing of drugs and toxins.

Thomas Knudsen

Developmental Systems Biologist
National Center for Computational Toxicology (NCCT)

Dr. Knudsen is a Developmental Systems Biologist at EPA's National Center for Computational Toxicology (NCCT), where he is a member of the ToxCast research team and leads the Virtual Embryo project. He obtained a PhD in Anatomy from Thomas Jefferson University in Philadelphia, postdoctoral training in Cell Biology at the Children's Hospital Research Foundation in Cincinnati and in Developmental Biology at Emory University in Atlanta. Prior to joining NCCT he was Professor of Molecular, Cellular and Craniofacial Biology at the University of Louisville, Birth Defects Center. Dr. Knudsen is a Past--President of the Teratology Society and is Editor in Chief of 'Reproductive Toxicology'.

Supplemental Information

The American Society for Cellular and Computational Toxicology (ASCCT)

Mission:

The ASCCT is a new scientific society which will provide 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 will facilitate 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:

- Our quarterly e-newsletter
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- 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 **fax:202.527.7435** **info@ascctox.org**

Help grow the society--become a member today!

Please complete this form and mail to:

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