New Technologies to Evaluate Organ-Specific Effects of Drugs and Chemicals

Tuesday, May 10 and Wednesday, May 11, 2022

University of North Carolina at Chapel Hill Friday Center
Chapel Hill, North Carolina

PROGRAM AND ABSTRACT BOOK

MEETING AGENDA

Tuesday, May 10 – Day 1

8:00 – 8:05 Welcome and Meeting Introduction
Ivan Rusyn (Texas A&M University)

8:05 – 8:50 ***Keynote: Quantitative Systems Toxicology: What Model Systems are Needed?***
Lorna Ewart (Emulate)

Plenary Session 1. New Models for Organ Injury Prediction and Assessment

8:50 – 10:00 **New Models for Evaluating Effects of Drugs and Chemicals on the Liver**

- 8:50 – 9:10 Perfused Microphysiological Systems
  Lawrence Vernetti (University of Pittsburgh)
- 9:10 – 9:30 Liver Spheroids and Micropatterned Cultures
  Salman Khetani (University of Illinois-Chicago)
- 9:30 – 10:00 Discussion of the Advantages/Disadvantages and Barriers to the Application of These Models or Data from These Models in Decision-Making

  Discussants**:** Vernetti, Khetani, Donna Mendrick (US FDA)

10:00 – 10:10 **Meeting Vendor Highlights**

10:10 – 10:20 Break

10:20 – 11:30 **New Models for Evaluating Effects of Drugs and Chemicals on the Blood-Brain Barrier**

- 10:20 – 10:40 Perfused Microphysiological Systems
  Michael Workman (Cedars-Sinai Medical Center)
- 10:40 – 11:00 Neurospheroid Models
  Lena Smirnova (Johns Hopkins University)
- 11:00 – 11:30 Discussion of the Advantages/Disadvantages and Barriers to the Application of These Models or Data from These Models in Decision-Making

  Discussants**:** Workman, Smirnova

11:30 – 11:40 **Meeting Vendor Highlights**

11:40 – 12:30 Lunch

12:30 – 1:40 **New Models for Evaluating Effects of Drugs and Chemicals on the Gut**

12:30 – 12:50 Intestinal Enteroids
Mark Donowitz (Johns Hopkins University)

12:50 – 1:10 Modeling the Gut Microbiome
Hyun Jung Kim (University of Texas-Austin)

1:10 – 1:40 Discussion of the Advantages/Disadvantages and Barriers to the Application of These Models or Data from These Models in Decision-Making

Discussants: Donowitz, Kim

1:40 – 2:50 **New Models for Evaluating Effects of Drugs and Chemicals on the Heart**

1:40 – 2:00 Perfused Microphysiological Systems
Kevin Healy (University of California-Berkeley)

2:00 – 2:20 High-Throughput *In Vitro* Models for Precision Medicine and Population-Based Testing
Alison Motsinger-Reif (NIEHS)

2:20 – 2:50 Discussion of the Advantages/Disadvantages and Barriers to the Application of These Models or Data from These Models in Decision-Making

Discussants: Healy, Motsinger-Reif, Weihsueh Chiu (Texas A&M University)

2:50 – 3:00 Break

3:00 – 4:10 **New Models for Evaluating Effects of Drugs and Chemicals on the Lung**

3:00 – 3:20 Perfused Microphysiological Systems
Dan Huh (University of Pennsylvania)

3:20 – 3:40 Air-Liquid Interface Models for Airway Safety Assessment
Alex Charlton (Syngenta)

3:40 – 4:10 Discussion of the Advantages/Disadvantages and Barriers to the Application of These Models or Data from These Models in Decision-Making

Discussants: Huh, Charlton

4:10 – 5:00 **Converting *In Vitro* Data to Human Exposure Levels: Advances in IVIVE**

4:10 – 4:30 Opportunities to Advance IVIVE with Physiological Organ-Specific Models
Weihsueh Chiu (Texas A&M University)

4:30 – 5:00 Discussion of IVIVE Science Needs of the New Toxicology Models

Discussants: Chiu, Barbara Wetmore (US EPA), John Wambaugh (US EPA)

5:00 – 6:30 Poster Session

Wednesday, May 11 – Day 2

8:30 – 8:45 Summary of Day 1
Thomas Knudsen (US EPA)

8:45 – 9:30 ***Keynote*: *Modeling Environment for Organ-Specific Computational Synthesis and Integration***
James Glazier (Indiana University-Bloomington)

Plenary Session 2. New Technologies for Organ Injury Prediction and Assessment

9:30 – 10:00 Novel Single Cell Sequencing-Based Technologies
James Cai (Texas A&M University)

10:00 – 10:30 Novel Imaging-Based Technologies (e.g., Bio-imaging)
Serguei Liachenko (US FDA)

10:30 – 11:00 *In Silico* Models of Organ-Specific Effects
Thomas Knudsen (US EPA)

11:00 – 11:15 Break

11:15 – 12:00 Discussion of the Advantages/Disadvantages and Barriers to the Application of These Technologies or Data from These Models in Decision-Making

Discussants**:** Cai, Knudsen, Donna Mendrick (US FDA)

12:00 – 1:00 Lunch

1:00 – 2:30 **Break-Out Group Discussions**

Breakout 1 (Co-Chairs: Donna Mendrick, US FDA; Katie Paul-Friedman, US EPA)

- What are future research needs in the areas of new technologies to evaluate organ-specific effects of drugs and chemicals?

Breakout 2 (Co-Chairs: Suzanne Fitzpatrick, US FDA; Sid Hunter, US EPA)

- What are the opportunities for the regulators to use new models and technologies in evaluating organ-specific effects of drugs and chemicals?

Breakout 3 (Co-Chairs: Michele Taylor, US EPA; Nicole Kleinstreuer, NIEHS/NTP)

- What “Big Data” streams are being used in toxicology and risk assessment, especially in the realm of organ-specific toxicity? What tools are generalizable to more traditional, yet complex data streams?

2:30 – 4:00 Break Out Group Reports and Discussion

4:00 Meeting Adjourned

MEETING SUPPORTERS

Our deepest appreciation to the following generous meeting supporters:

DIAMOND LEVEL

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

Scientific Liaison Coalition

PLATINUM LEVEL

CN-BIO

ELSEVIER

inSphero

NIH National Institute of Environmental Health Sciences

syngenta®

GOLD LEVEL

ACT | American College of Toxicology

NORTIS

Foundation for Chemistry
RESEARCH & INITIATIVES

ORGANIZING COMMITTEE MEMBERS

Chair: Ivan Rusyn, MD, PhD
Texas A&M University, College Station, TX

Co-Chair: Thomas B. Knudsen, PhD
US EPA/CCTE, Research Triangle Park, NC

Patrick Allard, PhD
University of California Los Angeles, CA

Jamie J. Bernard, PhD
Michigan State University, East Lansing, MI

Suzanne C. Fitzpatrick, PhD, SOT Council Contact
US FDA/CFSAN, College Park, MD

Jill A. Franzosa, PhD
US EPA/CCTE, Research Triangle Park, NC

Kevin S. McDorman, DVM, PhD, DACVP
Charles River Laboratories, Frederick, MD

Donna L. Mendrick, PhD
US FDA/NCTR, Silver Spring, MD

Xi Yang, PhD
US FDA, Silver Spring, MD

POSTER ABSTRACTS:

Alternative Models

1. Utilizing Multiomics Systems Biology Tools to Understand Toxicology

Thomas Kowal-Safron, Zacary Zamora, Yen-Wei Chen, Emily Quinto, Karen Garcia, Xia Yang

University of California, Los Angeles, USA

Abstract

Environmental toxicants have the potential to perturb many molecular pathways across tissues and organ systems and confer health risks. However, our understanding of toxicants at the systems level is currently lacking, preventing effective strategies to counteract toxicity. In this work, computational analysis of existing omics datasets and *de novo* experimental data generation are combined to produce novel tools and molecular understanding of an array of environmental toxicants. The past decades have generated an abundance of high throughput omics datasets to understand molecular pathways affected by environmental toxicants. However, a standardized, systematic data-driven analysis across diverse data platforms has not been conducted to directly compare toxicants and examine their associations with human diseases. A data-driven computational pipeline has been developed and applied to hundreds of existing omics datasets to establish a comprehensive gene signature database of environmental toxicants stratified by species, tissue, and dosage information, and further associate the gene signatures to human diseases. This information will be centralized and disseminated in an open-access online web server, ToxiOmics, to enable the community to query and understand the species-, tissue-, and dose-specific molecular pathways across toxicants. Upon completion of the database, we aim to leverage physiologically relevant 3D liver organoid systems, in conjunction with cutting-edge single cell multiomics technologies, to elucidate the molecular signatures of diverse environmental toxicants in a high-throughput manner. We will use these molecular signatures to predict cell-type specific toxicological mechanisms and further compare with liver signatures in ToxiOmics to understand similarities and differences between studies and model systems.

2. Comparing the Respiratory Burst *In Vivo*, *In Vitro*, and *Ex Vivo* After Exposure to Per- and Polyfluoroalkyl Substances

Drake Phelps, Haleigh Conley, M. Katie Sheats, Jeffrey Yoder

North Carolina State University, Raleigh, NC, USA

Abstract

The United States Environmental Protection Agency currently estimates that there are more than 12,000 per- and polyfluoroalkyl substances (PFASs), which are used to produce non-stick cookware, food contact materials, hydro- and oleophobic textiles, and more. Due to their unique chemistry, they are ubiquitous and persistent in the environment, making exposure to PFASs commonplace. It is estimated that 98% of Americans have detectable serum levels of multiple PFASs. These compounds have also been detected in wildlife, illustrating their wide-reaching impact. It is well established that these compounds are immunotoxic; however, previous research has focused largely on the effects of PFASs on the adaptive immune system, leaving a knowledge gap on what is known about the effects of these compounds on the innate immune system. To bridge this gap, we utilized an *in vivo* larval zebrafish model, an *in vitro* human neutrophil-like cell culture model, and primary neutrophils exposed to PFASs *ex vivo* to investigate innate immune function after exposure to environmentally relevant PFASs. The respiratory burst was measured as a functional readout of innate immune function. Neutrophils induce microbicidal reactive oxygen species through the respiratory burst to defend the host against pathogens. Data show that some PFASs are capable of inhibiting the respiratory burst *in vivo, in vitro*, and *ex vivo*. Potency was similar among the model systems, indicating potential evolutionary conservation. Current studies are exploring whether exposure to PFASs confers susceptibility to infectious disease, and what mechanisms may be responsible for this immunosuppressive phenotype.

3. A Population Screen of Chemical Toxicity Using High-Throughput Phenotypic Profiling (HTPP) in Diversity Outbred Neural Progenitor Cells

Alison Harrill[1], Johanna Nyffeler[2], Clinton Willis[1], Camille Michelon[3], Ihab Daou[3], Dahea You[4], Ted Choi[3], Megan Culbreth[1], Jiu-Hua Hsieh[4], Joshua Harrill[1]

[1]US EPA, Durham, NC, USA. [2]ORISE, Oak Ridge, TN, USA. [3]Predictive Biology Inc., Carlsbad, CA, USA. [4]NIEHS, RTP, NC, USA

Abstract

Diversity Outbred (DO) mice that mimic human heterozygosity and are highly genetically diverse provide an ideal resource to interrogate toxicity responses unique to sensitive sub-populations. Members of the Tox21 Consortium utilized female DO neural progenitor cell lines, derived from genetically unique mice, coupled with the US EPA's established high throughput phenotypic profiling (HTPP) assay to interrogate interindividual variability in developmental neurotoxicity susceptibility. DO cell lines were exposed to 12 chemicals (0-100 uM, 8 dose levels, N=3) for 24 h and labeled with 6 fluorescent probes to visualize multiple organelles, including nucleus, nucleoli, endoplasmic reticulum, golgi, cytoskeleton, plasma membrane and mitochondria. 1,300 phenotypic measures were quantified from confocal imaging and well-level data were normalized to each cell line's DMSO control, with phenotypic profiles assessed at concentrations at the cytotoxicity LOEL and below. Phenotypic response across lines was quantified using global Mahalanobis distance and a benchmark concentration (BMC) associated with the threshold for phenotypic effects was determined for each cell line. In an initial analysis phase using 98 DO cell lines, chemicals displaying varied degrees of inter-individual variability in BMC included 4-nitrosodiphenylamine, 5-fluorouracil, dieldrin, hexachlorophene; methylmercuric(II) chloride, rotenone, tebuconazole, BDE-99, captan, and triphenyl phosphate isopropylated. While HTPP testing of additional DO cell lines is ongoing toward assessing chemical modes of action operant in susceptible individuals, these initial data represent an important first step toward a high content screening strategy that may inform population variability in dose and phenotypic response. This abstract does not necessarily reflect US EPA policy.

4. Engineering Human Neural Organoids to Explore Impaired Neurogenesis Induced by Arsenic

Xian Wu, Darlene Dixon, Erik Tokar

DNTP/NIEHS, DURHAM, USA

Abstract

Modeling brain development is challenging due to complexity of the organ. Recent advances in human pluripotent stem cell (PSC)-derived brain-like organoids together with 2D cellular assays have the potential to enhance our understanding of the mechanisms of developmental neurotoxicity (DNT). Exposure to environmental contaminant Arsenic (As) is associated with DNT-related diseases. However, mechanisms of As on DNT are not well-defined. Here, we used 3D PSC-derived embryoid bodies (EBs) to recapitulate early embryogenesis and neurogenesis events before neural induction, and EB-derived neural organoids to mimic neural development *in vivo*. A 7-day exposure to a human-relevant dose (0.5 μM) of As increased ectoderm differentiation within the EBs through upregulated expression of PAX6, SOX1, COL2A1. Histological staining of As-treated EBs showed neural rosette structure disruption. The IPA-identified top 5 pathways affected by As were CREB signaling in neurons, neuronal synapse pathway, GABA receptor pathway, synaptogenesis and axonal guidance signaling. GO enrichment analysis found G-protein signaling was suppressed and WNT, Notch, and FGF signaling pathways were all inhibited by As treatment. RNAseq analyses were confirmed by real-time qPCR, which found As-inhibited mature neural cells (MAP2, vGLUT2) and astrocytes markers (GFAP). Inhibition of neuronal gene expression was also confirmed in a neurite outgrowth assay that mimics nerve growth *in vivo* and in the disrupted neural rosette and neuropil structures in day 40 organoids. In conclusion, the results described herein show that this EB and neural organoid 3D model system can provide valuable insights into the cellular events and molecular mechanisms of As-induced DNT.

5. Evaluating the Dermatotoxicity of Mechlorethamine in Both *In Vivo* And *In Vitro* Models

Trishaal J.R. Rao, Ganming Mao, Blase Billack

St. John's University, New York, USA

Abstract

Sulfur Mustard (SM) is a chemical warfare agent that was introduced on the battlefield more than 100 years ago. To the present time, there is no antidote to poisoning by SM. Mechlorethamine (HN2) is a derivative of SM which is used in anticancer therapy. Dermal exposure of HN2 is associated with tissue blistering (vesication) which limits its clinical usefulness. A major purpose of the present study was to investigate the dose dependent dermatotoxicity of HN2 using an *in vivo* mouse ear vesicant model (MEVM). The ears of male and female C57BL/6 mice were exposed to increasing doses of HN2 (0.125, 0.250, 0.500 & 1.000 umol/ear) or vehicle (DMSO). Mice were then euthanized 24 hr following exposure. HN2-exposed ears showed an increase in wet weights, morphometric thickness and MMP-9+ cells (tissue remodeling marker) at all test doses when compared to control. As an alternative to the MEVM, we next evaluated tissue damage caused by HN2 in a reconstructed human skin full thickness model. A preliminary study was performed using samples of T-skin™ treated topically with HN2 (1.0 umol/tissue) or DMSO and biopsied 1 or 4 hr after exposure. H&E stained samples of HN2-treated skin showed signs of tissue injury compared to untreated samples or those treated with DMSO. Decreased immunostaining of collagen IV, a marker for dermal-epidermal junction was present in the HN2 treated tissues. Further developments in reconstructed 3D tissues are required to better mimic inflammatory responses which can only be observed in animals and humans for now.

6. *In Vitro* Irritation Testing of Non-Extractable Medical Devices with the ISO 10993-23:2021 Standard Protocol

Christian Pellevoisin[1], Kelly Coleman[2], Sebastian Hoffmann[3]

[1]Urbilateria Consulting, Lyon, France. [2]Medtronic plc, Minneapolis, USA. [3]seh consulting + services, Paderborn, Germany

Abstract

Irritation testing is an integral part of the biocompatibility assessment of medical devices and has historically been conducted on animals, either by direct contact or with polar and non-polar solvent extracts. In 2018, an ISO-sponsored interlaboratory validation study demonstrated that two reconstituted human epidermis (RhE) assays, which were adapted from validated methods used for industrial chemicals, produced results essentially equivalent to those obtained with *in vivo* tests. This led to the publication of the ISO 10993-23:2021 standard on irritation testing, which states that RhE assays are now the preferred method. The 2018 validation study evaluated strong irritants, so we tested nine mild irritants (GHS Category 3), neat and spiked at different concentrations into medical device extracts, per ISO 10993-23. The results substantiated the applicability of RhE assays for evaluating mild irritants in medical device extracts. In addition, the 2018 validation study tested solid extractable medical device materials but did not consider non-extractable medical device materials (e.g., creams, gels, or sprays). By testing nine marketed non-extractable materials, either neat or spiked with irritants, we also confirmed that RhE assays are readily applicable to such medical device materials.

7. Pre-Validation of SENS-IS Assay for *In Vitro* Skin Sensitization Testing of Medical Devices

Christian Pellevoisin[1], Françoise Cottrez[2], Jenny Johansson[3], Emma Pedersen[4], Kelly Coleman[5], Herve Groux[2]

[1]Urbilateria Consulting, Lyon, France. [2]ImmunoSearch, Grasse, France. [3]RISE Research Institutes of Sweden, Borås, Sweden. [4]Key2Compliance, Göteborg, Sweden. [5]Medtronic plc, Minneapolis, USA

Abstract

According to ISO 10993-1:2018, the skin sensitization potential of all medical devices must be evaluated, and for this endpoint ISO 10993- 10:2010 recommends the use of *in vivo* assays. The goal of the present study was to determine if the *in vitro* SENS-IS assay could be a suitable alternative to the current *in vivo* assays. The SENS-IS assay uses the Episkin Large and SkinEthic RHE reconstructed human epidermis models to evaluate marker genes. In our study, the SENS-IS assay correctly identified 13 sensitizers spiked in a non-polar solvent. In a subsequent analysis six medical device silicone samples previously impregnated with sensitizers were extracted with polar and non-polar solvents. The SENS-IS assay correctly identified five of these extracts, while a sixth extract, which contained the weak sensitizer phenyl benzoate, was classified as negative. However, when this extract was concentrated, or a longer exposure time was used, the assay was able to detect phenyl benzoate. The SENS-IS assay was transferred to a naïve laboratory which correctly identified sensitizers in six blinded silicone samples, including the one containing phenyl benzoate. In light of these results, we conclude that the SENS-IS assay is able to correctly identify the presence of sensitizers in medical devices extracts.

8. Cell-Free DNA is Predictive of Differentiation and Toxicity in Cardiac Organoids

Brian Silver, Kevin Gerrish, Erik Tokar

NIEHS, Durham, USA

Abstract

Drugs and chemicals can have many damaging off-target effects, such as cardiac injury. In addition, exposure to certain toxicants during development can cause fetal abnormalities, including heart defects. However, the cardiotoxicity of many compounds, particularly during developmental stages, remains unclear. Advances in efficient, non-invasive monitoring strategies are therefore needed to improve the safety and prediction of cardiotoxicants. Liquid biopsies are valued for their potential as non-invasive diagnostic tools for early detection of disease. Cell-free DNA (cfDNA) which refers to free-floating DNA in the bloodstream or extracellular media, is believed to be generated largely by apoptotic cells. Several parameters including concentration, fragment size, and sequence can be used to characterize cfDNA, and infer information about the cells from which it originated. However, to accurately deduce cellular health from cfDNA, we need a more thorough mechanistic understanding of how nucleic acids are released in response to events at the cellular level. Here, we used cardiac organoids to identify key changes in cfDNA associated with cardiac development and toxicity. We observed that the concentration of cfDNA fluctuates during differentiation. Also, we observed alterations in the prevalence of specific genomic regions in cardiac organoid-derived cfDNA during development. Specifically, the fraction of mitochondrial cfDNA was regulated during organoid development, suggesting the possibility that mitochondrial dynamics and maturation at the tissue level are reflected in cfDNA. Our current efforts include identifying additional cfDNA sequences that are altered during cardiac differentiation and understanding how drugs and toxins impact the cfDNA profiles of mature and developing cardiac tissues.

9. Mechanism Of Hypoxia-Induced Inhibition of Steroid Biosynthesis in MA-10 Leydig Cells

Alanna McFail

Campbell University, Lillington, USA

Abstract

Testicular hypoxia has been shown to exert an inhibitory effect on luteinizing hormone (LH)-stimulated Leydig cell steroid biosynthesis. However, the molecular mechanism of its inhibitory effects remains unclear. In the present study, we examined functional changes in the steroidogenic pathway components and the mitochondrial function that result from exposing MA-10 mouse tumor Leydig cells to hypoxia and relate these changes to reduced progesterone formation. Exposure to hypoxia resulted in decreased progesterone biosynthesis by MA-10 cells in response to LH, dcAMP, or 22HC. The loss-of-ability of the Leydig cells to produce progesterone was associated with decreased CYP11A1 enzymatic activity as evidenced by the failure of 22HC to overcome the inhibitory effect of hypoxia, and with independence of STAR. Hypoxia condition also caused a decrease in electron flow through the electron transport chain in the mitochondria, and this was correlated with increased generation of mitochondrial superoxide. Together, these observations suggest that exposure of MA-10 cells to hypoxia can alter the ability of the cells to produce progesterone via inhibition of the conversion of cholesterol to pregnenolone by CYP11A1 perhaps resulting from increased oxidative stress.

10. The 3D Liver Spheroid DILI Model as a Pragmatic Industrial Approach to Enable Pharma-Internal Go/No-Go Decisions

Armin Wolf[1], Friederike Wenz[1], Monika Tu[2], Natalia Zapiorkowska[1], Bruno Filippi[1]

[1]InSphero AG, Schlieren, Switzerland. [2]Insphero AG, Schlieren, Switzerland

Abstract

Microphysiological systems (MPS) have been raising high expectations of their regular use in everyday industrial life for several years. Studies showed the value of these new methods although few make it into the industrial workflow. Drug-induced liver injury (DILI) is a common reason for the failure of new drug candidates in preclinical and clinical development. 3D liver microtissues (MT) are superior DILI predicters than 2D liver cell cultures (Procter W., et al. Arch Tox. 2017). 3D liver models are primary liver cell co-cultures of a 10-donor pool (i.e. hepatocytes, endothelial and Kupffer cells) with essential structural and functional features of the native liver. 3D liver models are amenable to large-scale screening through highly standardized production in AkuraTM 384-well plate format. The present data show why 3D liver models meet the needs of the drug development process from early discovery to clinical development. A compound set selected according to the IQ-MPS consortium criteria was evaluated by a two-tier testing strategy. First, the effect of each compound on cellular ATP, LDH leakage and albumin secretion was measured. Subsequently, the effect of specific “sensitizer” (e.g. BSO, LPS) was evaluated on Tier 1 negative DILI candidates. It turned out that the sensitivity of the model is significantly increased. The flexible, fast and cost-effective usage of the 3D MTs allows to generate big high-quality DILI datasets which are important in each state of the drug development process. This leads to high level of pharma-compatibility and thus enable internal go/no-go decision.

Data Analysis

11. An Integrated and Human-Relevant Translational Toxicology Paradigm for Environmental Cardiovascular Hazard Assessment

Rachel Dee[1], Scott Auerbach[1], Brian Berridge[1], Kevin Dreher[2], David Gerhold[3], Nicole Kleinstreuer[1], Shagun Krishna[1], Kelly Shipkowski[1], Sreenivasa Ramaiahgari[1], Xian Wu[1], Brandy Beverly[1]

[1]National Institute of Environmental Health Sciences, Durham, USA. [2]North Carolina State University, Raleigh, USA. [3]National Center for Advancing Translational Sciences, Bethesda, USA

Abstract

Cardiovascular (CV) disease remains the most significant global cause of morbidity and mortality in humans. Lifestyle choices and genetics are clearly key contributors but cannot alone or even in combination account for all the risk associated with the development of CV disease. Environmental exposures are presumed to contribute and, in some cases have compelling evidence to support that likelihood. The NIEHS Division of the National Toxicology Program has initiated a Cardiovascular Health Effects Innovation initiative that aims to design and test a translational toxicology pipeline of hazard assessment capabilities to rapidly and accurately identify environmental agents that contribute to CV diseases. Our approach enables the development of evidence-based capabilities, beginning with *in silico* QSAR modeling and medium to high throughput bioactivity screening, continuing through simple and complex *in vitro* confirmatory assays, and culminating with holistic *in vivo* assessment in animal models enhanced for evaluation of fundamental physiologic measures. Early predictions based on *in silico* models and *in vitro* bioactivity will be qualified in progressively complex assay systems allowing us to build confidence in early pipeline steps, assess model applicability, and identify capability development needs. The assay systems used will be aligned to known human CV failure modes and reflect human biology as much as the complexity of the system permits with a goal of optimizing the translational relevance of the outcomes. We believe this effort will support our ability to identify environmental contributors to CV diseases with high prevalence in society today.

12. Developing *In Vitro* Assay Annotations to Provide Context and Facilitate Interpretation Toward Toxicological Endpoints

Agnes Karmaus[1], Patricia Ceger[1], John Rooney[1], Shannon Bell[1], David Allen[1], Nicole Kleinstreuer[2]

[1]ILS an Inotiv Company, RTP, USA. [2]NIH/NIEHS/DNTP/NICEATM, RTP, USA

Abstract

In vitro assays, especially high-throughput screening (HTS) assays, can generate an abundance of mechanistic data to inform on chemical effects on biochemical endpoints and molecular and cellular signaling pathways. However, interpreting HTS data in the context of organism-level outcomes can be challenging. Assay descriptions are often focused on technological features such as cell line, receptor type, and reporter used. Contextualization can be further complicated when cell lines have a transfected target molecule from a different species, or when the assay's measured endpoint (e.g., a change in the expression of a gene transcript) is different from the process the assay is intended to inform on (e.g., activity of a receptor that mediates the expression of that gene transcript). For stakeholders unfamiliar with HTS assays, the lack of context may lead to either misinterpretation of the data or a hesitancy to use the data at all. This presentation describes how annotating HTS assays with complementary technological and biological target pathway information can provide context needed to improve accessibility of these data to a broader range of stakeholders. Focusing on the curation of HTS data within the National Toxicology Program's Integrated Chemical Environment (https://ice.ntp.niehs.nih.gov/), we will demonstrate the use of knowledge organization systems and controlled terminology to facilitate human and machine-accessible data interpretation in support of chemical evaluation for toxicological hazard characterization. This project was funded by the National Institute of Environmental Health Sciences, National Institutes of Health, under Contract No. HHSN273201500010C.

13. A Benchmark Concentration Analysis Method for Larval Zebrafish Locomotor Response Data Using ToxCast Pipeline Software

Zachary Rowson[1,2], Rhyne Woodrow Setzer[1,3], Katie Paul Friedman[1], Richard Judson[1], Stephanie Padilla[1]

[1]Center for Computational Toxicology and Exposure, Office of Research and Development, US Environmental Protection Agency, Research Triangle Park, NC, USA. [2]Oak Ridge Institute for Science and Education, U.S. Department of Energy, Oak Ridge, TN, USA. [3]Emeritus Scholar, Research Triangle Park, NC, USA

Abstract

Larval zebrafish (Danio rerio) locomotor response (LMR) behavior may inform developmental neurotoxicity hazard. This work proposes a method for evaluating chemical activity in the LMR assay that produces probabilistic representations of the likelihood of chemical activity and estimates point of departure (POD) using Benchmark Concentration (BMC) analysis. To allow for BMC analysis, complex time-series data derived from zebrafish larvae exposed during development to 61 chemicals were summarized into 16 endpoints. Concentration-response curves were fit using the new ToxCast Pipeline curve-fitting and hit-calling utility, tcplfit2, a publicly available R package. Chemicals were clustered by their activity across all 16 endpoints. The analysis approach was compiled into a new R package, gabi, which summarized and transformed longitudinal data prior to application of tcplfit2. Of the 61 assayed chemicals, 22 were found to be active in at least one endpoint. No more than 7 chemicals were active in any given endpoint and all endpoints other than those describing jerk in Light or Dark found at least two chemicals to be active. Characterization of chemical activity in multiple endpoints better captured changes in the LMR that were implied by plots of the longitudinal data. This analysis method quantifies the significance of behavioral changes and estimates BMC which could be used to compare chemical potency across assays. It is possible that production of activity profiles for large sets of chemicals could establish common behavioral phenotypes elucidating common modes of action. This abstract does not necessarily reflect U.S. EPA policy.

14. *In Vitro-In Vivo* Extrapolation (IVIVE) For Neurodevelopment: Toxicokinetics and *In Vitro* Point of Departure Evaluation of Putative Developmental Neurotoxicants

Anna Kreutz[1,2], Timothy Shafer[3], Katie Paul-Friedman[3], John Wambaugh[3], Barbara Wetmore[3]

[1]ORISE, Durham, USA. [2]NIEHS, Durham, USA. [3]US EPA, Durham, USA

Abstract

In vitro new approach methodologies (NAMs) that screen for developmental neurotoxicity (DNT) hold great potential over traditional DNT studies for risk assessment. However, alongside additional uncertainties, these assays lack two key barriers that modulate concentrations at the site of brain development—the fetoplacental and blood brain barriers—leading to gaps in translating *in vitro* potency values to *in vivo* concentrations in the brain during windows of susceptibility for neurodevelopment. To address this, we developed a customized IVIVE approach. To estimate human equivalent doses that could elicit DNT-relevant bioactivity, IVIVE was performed using physiologically-based pharmacokinetic (PBPK) modeling during these windows of susceptibility—15 and 24 gestation weeks, and 2 weeks and 1 year of age. This approach incorporated *in vitro* toxicokinetic data to predict maximal concentrations (Cmax) in target tissues for chemicals screened in DNT NAMs. Administered equivalent doses (AEDs) were calculated from Cmax predictions and bioactivity data using reverse dosimetry. For chemicals with *in vivo* DNT data, AEDs overlapped with doses that elicited *in vivo* DNT effects at comparable lifestages, suggesting this approach holds potential for setting testing priorities. Incorporation of external exposure allowed derivation of bioactivity:exposure ratios—an ad hoc margin of exposure estimate for risk prioritization. As with most PBPK models, this DNT-IVIVE approach is modularized to allow incorporation of additional exposure routes (e.g., lactation) and metabolism (e.g., ontogenetics), with future plans to incorporate transporter data to further refine target tissue estimations across multiple lifestages. This abstract does not necessarily represent the views or policies of EPA.

15. Fit for Purpose Evaluation of *In Vitro* Assays for IVIVE

Xiaoqing Chang[1], Shannon Bell[1], Jaleh Abedini[1], David Allen[1], Nicole Kleinstreuer[2]

[1]Inotiv-RTP, Research Triangle Park, USA. [2]NIH/NIEHS/DNTP/NICEATM, Research Triangle Park, USA

Abstract

In vitro assays are a quick and often cost-effective way to generate information on how a chemical might interact with biological systems. Compared to *in vivo* studies, which integrate several underlying processes and observe apical endpoints, *in vitro* assays are typically mechanistic and inform a specific biological process. When combined with *in vitro* to *in vivo* extrapolation (IVIVE), which allows estimation of *in vivo* external exposures yielding internal concentrations equivalent to *in vitro* bioactive concentrations, *in vitro* assays can also provide insight on safe exposure levels. User-friendly tools now exist that facilitate access to data based on alternative methods and make IVIVE analysis more widely accessible. One of these tools is the Integrated Chemical Environment (ICE: https://ice.ntp.niehs.nih.gov/). Whatever tool is used, understanding the modeling assumptions, application scenarios, and limitations of this approach is important for obtaining the desired outcome. This presentation will present the criteria and considerations for conducting IVIVE analysis with a focus on *in vitro* assay selection and IVIVE results interpretation. Case studies using ICE will be provided to highlight the impacts of decisions on input factors such as bioactivity concentration metrics and *in vitro* assay type on IVIVE outputs. The presentation will also discuss strategies for *in vitro* assay selection under different fit-for-purpose scenarios, as well as possible challenges when relating IVIVE outputs to *in vivo* toxicity data. This project was funded by the National Institute of Environmental Health Sciences, National Institutes of Health, under Contract No. HHSN273201500010C.

16. Integrating Parameter Uncertainty in PBPK Modeling

David Hines[1], Jaleh Abedini[1], David Allen[1], Nicole Kleinstreuer[2], Kamel Mansouri[2]

[1]Inotiv-RTP, Morrisville, USA. [2]NIH/NIEHS, Durham, USA

Abstract

Physiologically based pharmacokinetic (PBPK) models combine physiological and chemical-specific parameters to simulate forward and reverse dosimetry, and thus are useful for linking internal concentrations with external doses. Chemical-specific experimental parameter data are needed to run these simulations. When these data are unavailable, quantitative structure-activity relationship (QSAR) models can be used to generate parameter predictions to fill these data gaps. However, the accuracy of QSAR parameter predictions depends on the resemblance of a target chemical to the training set of the model, among other parameters. Understanding how the uncertainty associated with this accuracy affects PBPK model output is essential for interpreting results. We evaluated how QSAR output ranges based on nearest neighbor analysis informs uncertainty in PBPK model predictions. Specifically, parameter value combinations were systematically evaluated based on the full range of parameter values for intrinsic clearance, fraction unbound in plasma, pKa, Henry's law constant, and octanol:water partition coefficient (logP) using the oral gavage and inhalation PBPK models from the U.S. Environmental Protection Agency's httk package. The parameters that influence the maximal and minimal values of response variables were then identified. In this presentation, we will demonstrate how the QSAR-based uncertainty ranges of PBPK model outputs can affect tissue concentration and area-under-the-curve ranges generated by forward dosimetry, and margin of exposure assessments informed by *in vitro* to *in vivo* extrapolation. This project was funded by the National Institute of Environmental Health Sciences, National Institutes of Health, under Contract No. HHSN273201500010C.

17. Incorporating Microphysiological Systems into *In Silico* Knowledge Platforms to Support Translation and Decision Making: A Case Study with Cardiotoxicity

Adrian Fowkes, Anax Oliveira

Lhasa Limited, Leeds, United Kingdom

Abstract

Microphysiological systems herald new avenues for toxicity assessments given their ability to recapitulate target systems *in vitro*, which can provide higher throughput and mechanistic insight compared to traditional forms of toxicity testing. Acceptance and re-use of novel assays can be hampered by the lack of data integration, as the protocols and assay results needed to support translation during safety assessments may not be readily available to users. Adverse outcome pathways (AOPs) describe measurable events in pathways leading to toxicity and thus provide a framework to allow users to interrogate assay results and relationships in the context of biological mechanisms. To demonstrate the utility of such an AOP framework, known molecular initiating events (MIEs) for cardiotoxicity, relevant assays and their results were organised into an AOP-oriented data model. Assay data for the MIEs was used to train quantitative structure-activity relationship models, which were used to fill data gaps and identify modes of action for compounds flagged in more complex assays. Organisation of assays into the AOP framework allowed for statistical relationships between assays results to be identified and placed alongside biological plausibility of toxicity mechanisms. The data could be queried from the biological perspective, allowing users to examine assay relationships at the target or pathway level. In addition, the chemical perspective could be queried by filtering the data for specific chemotypes. Overall, the approach was able to store assay protocols and the results for reference molecules, allowing users to make informed assay selection during their safety assessment strategy.

18. Engineering a Computable Epiblast for *In Silico* Gastrulation and Predictive Modeling of Developmental Toxicity with *In Vitro* Data from the ToxCast Stem Cell Assay

Kaitlyn Barham[1,2], Richard Spencer[3], Thomas Knudsen[2]

[1]Oak Ridge Associated Universities, Oak Ridge, USA. [2]U.S. Environmental Protection Agency, Durham, USA. [3]General Dynamics Information Technology, Falls Church, USA

Abstract

New Approach Methods (NAMs) for assessing chemical effects on prenatal development with less reliance on animal testing is important for predictive toxicology at the USEPA [https://www.epa.gov/chemical-research/comptox-chemicals-dashboard]. The ToxCast portfolio provides a vast resource of *in vitro* data for *in silico* modeling of toxicity, including data on over 1000 chemicals in a human pluripotent stem cell (hPSC) assay predicting developmental toxicity with ~80% balanced accuracy [Zurlinden et al. 2020]. The mammalian epiblast is the embryonic structure most closely reflected by the developmental potential of hPSCs *in vitro*; therefore, a computer model recapitulating cellular dynamics of the epiblast *in silico* facilitates tracking of normal versus adverse developmental trajectories during gastrulation - a critical process where the embryonic body plan is decoded from the genome. We engineered a fully computable epiblast model in the www.compucell3d.org modeling environment that recapitulates development of the primitive streak and subsequent epithelial-mesenchymal transition from epiblast stem cells. The model self-organizes progenitors destined for different mesodermal domains (chordamesoderm, paraxial, lateral plate, extraembryonic) formed during mouse and human gestation. The *in silico* epiblast provides a quasi-normal simulation of gastrulation under control of morphogenetic signals (e.g., WNT, FGF, Nodal) that can be tweaked with ToxCast bioactivity data. This agent-based model uniquely integrates chemical bioactivity data with cell signaling networks and developmental processes driving gastrulation for mechanistic prediction of altered phenotypes. Case studies are underway using ToxCast portfolio chemical effects data to virtually screen complex interactions between genetic and/or environmental stressors. *This abstract does not necessarily reflect Agency policy.*

19. Identification of Chemicals Associated with Retinoid Signaling Pathway Disturbance and Skeletal Dysmorphogenesis Through Predictive Computational Toxicology Models

Jocylin Pierro

US EPA, Research Triangle Park, USA

Abstract

All-trans retinoic acid (ATRA) gradients determine skeletal patterning morphogenesis and can be disrupted by diverse genetic or environmental factors, leading to fetal skeleton malformations. Here, a data-driven model was constructed to identify chemicals associated with both ATRA pathway bioactivity and prenatal non-human, mammalian skeletal defects. We classified altered skeletal phenotypes in prenatal developmental toxicity studies in ToxRefDB and/or ToxCast high-throughput screening (HTS) and identified 370 chemicals associated with alterations. Defects were organized into four skeletal phenotype groupings: cranial, post-cranial axial, appendicular, and non-specified skeletal defects. To build a multivariate statistical model, HTS results from >8,070 chemicals in ToxCast/Tox21 across 10 *in vitro* assays, representing key nodes in the retinoid signaling system were evaluated and compared to candidate reference chemicals for *in vitro* testing. A set of 48 chemicals were identified for constructing data-driven models to link this *in vitro* data with adverse skeletal outcomes for computational modeling. Adverse Outcome Pathway (AOP) frameworks for ATRA metabolism, signaling, and homeostasis allow for the development of new approach methodologies (NAMs) to improve predictive toxicology without animal experimentation. Our findings guided the development of potential AOPs (pAOPs) and will further advance our dynamic mechanistic modeling to strengthen evidence for causality. Furthermore, NAMs identified 20 chemicals without previous evidence of retinoic acid pathway disturbance and skeletal defects association. Extrapolations of these vertebrate findings shed light on potential avenues for new mechanistic discoveries related to retinoic acid pathway disruption and associated skeletal dysmorphogenesis in human fetuses. This abstract does not represent the views of EPA.

Microphysiological Systems

20. Feto-Maternal Interface Organ on Chip (FM-OOC) for Rapid Evaluation of Chemical Hazards That May Cause Preterm Birth

Sungjin Kim[1], Lauren Richardson[1,2], Enkhtuya Radnaa[2], Zunwei Chen[1], Ivan Rusyn[1], Ramkumar Menon[2], Arum Han[1]

[1]Texas A&M University, College Station, USA. [2]University of Texas Medical Branch at Galveston, Galveston, USA

Abstract

Human labor is associated with feto-maternal-derived signals that coordinate to initiate delivery. Exposure to environmental chemicals can prematurely trigger labor-initiating signals at the feto-maternal interface (FMi: decidua, amniochorion), leading to spontaneous preterm birth (PTB). Testing the association between environmental chemical exposure and PTB is difficult due to many *in vivo* or *in vitro* limitations. Physiological organ-on-chips (OOCs) are potential alternatives for studying both potential hazards and mechanisms leading to PTB. This study tested the FMi-OOC that incorporates maternal decidua cells and three different fetal cells (chorion, amnion mesenchymal, and amnion epithelial cells) as a model for rapid evaluation of chemical hazards that may cause preterm birth. As a case scenario, we evaluated the effect of maternal exposure to cadmium (Cd), an environmental toxin, using FMi-OOC. Cd transport through the FMi and its impact on cell cycle, cell death, and inflammation were analyzed. Cd treatment resulted in significant cell death and a pro-inflammatory environment in the maternal decidua, but had minimal effect on the fetal chorion cells, and no effect in the fetal amnion cells compared to controls. The maternal response, but lack of fetal response, indicates that Cd-mediated adverse effects originate from maternal pathophysiology rather than fetal-derived triggers of preterm labor. Overall, this study demonstrates that the FMi-OOC can indeed predict the response of FMi upon exposure to chemicals, opening the possibility for using OOC models for environmental chemical screens.

21. Investigation of Intestinal Permeability in three Microphysiological systems

Haley Moyer[1], Leoncio Vergara[2], Alan Valdiviezo[1], Courtney Sakolish[1], Weihsueh Chiu[1], Clifford Stephan[2], Ivan Rusyn[1]

[1]Texas A&M University, College Station, TX, USA. [2]Institute of Biosciences and Technology, Texas A&M University, Houston, TX, USA

Abstract

This poster details the work on testing options for small intestine microphysiological systems (MPS) as part of the tissue chip testing Consortium at Texas A&M University (TEX-VAL Consortium). The Consortium is a collaboration of pharmaceutical and consumer goods companies, federal agencies, and a trade association with the Texas A&M University Tissue Chip Testing Center. Researchers at Texas A&M conduct testing, develop detailed protocols, and report results to Consortium members and the general scientific audience. One of the areas of interest across the members of the Consortium is the small intestine. The small intestine is a key site for absorption, and it is important to understand transport and permeability of small molecules in MPS with and without fluid flow. Previous studies have investigated small molecule permeability in Caco-2 cells, which has been previously accepted as standard in *in vitro* intestinal permeability studies. Additional studies using excised intestinal tissue segments have demonstrated differences in the rate of permeability between regions of the small intestine. In this study, we investigated apparent permeability, transport direction, and barrier function in three cell types (Caco-2, and human intestinal enteroid cells isolated from jejunal (J2) and duodenal (D109) tissues) using four compounds (caffeine, propranolol, indomethacin and fexofenadine). Studies were carried out in the Mimetas 3-lane Organoplate ® (MPS), CN-Bio PhysiomimixTM TC-12 (MPS), and static transwell systems. Comparative studies of differences in permeability between the cell types and model systems are highly informative for the potential end-users of MPS.

22. Role of Shear Stress in Renal Xenobiotic Transporter Function in a Microphysiological System

Courtney Sakolish, Ivan Rusyn

Texas A&M University, College Station, USA

Abstract

This poster details the testing of various cell options for a renal proximal tubule microphysiological system (MPS) that was part of the tissue chip testing Consortium at Texas A&M University (TEX-VAL Consortium). The Consortium is a collaboration of pharmaceutical and consumer goods companies, federal agencies, and a trade association with the Texas A&M University Tissue Chip Testing Center. One of the areas of interest across the members of the Consortium is the kidney proximal tubule. This region of the nephron is responsible for reclamation of water, salts, sugars and other small molecules from the glomerular filtrate, as well as secretion and reabsorption of xenobiotics. The activity of these xenobiotic transporters can lead to accumulation of compounds in the tubule, and result in drug-induced kidney injury. In this study, we investigated transporter function in 6 sources of human RPTECs (TERT1-basal, -OAT1, -OCT2, -OAT3 cell lines, and primary cells from 2 unique donors). These studies were carried out in a Mimetas 3-lane Organoplate® (MPS), or traditional 2D culture (384 well plate comparator) to study the effects of dynamic conditions on transporter function. Here, we explored pAH secretion (OAT-1, MRP2), EAM-1 uptake (OCT-2), 6-CF uptake (OAT-1), Calcein AM efflux (P-gp), CMFDA efflux (MRP2/4), and 6-NBDG influx (SGLT2) in the presence or absence of transport inhibitors. We demonstrated significant differences in handling and transporter function with these substrates between static (384 well plate) and fluidic (OrganoPlate® MPS) conditions, as well as between cell sources, highlighting the importance for “fit for purpose” modeling.

23. An *In Vitro* 3D Model of Human Renal Proximal Tubule for Nephrotoxicity Screening Studies

Adam Pearson, Stephen Ferguson

National Institute of Environmental Health Sciences (NIEHS), Durham, USA

Abstract

Nephrotoxicity from xenobiotics is a major cause of kidney disease and a common reason for drug development failure. Proximal tubule (PT) cells are the most frequent site of damage as they transport and metabolize xenobiotics, leading to intracellular accumulation of reactive metabolites. Typically, toxic insults impair PT solute reabsorption and disrupt essential nutrient homeostasis, causing negative health effects. While animal experiments can detect mid-to-late-stage kidney damage, our ability to identify and mechanistically understand early-stage nephrotoxicity remains insufficient. Furthermore, species differences and ethical considerations limit the utility of animal models for human translation. *In vitro* systems enable detailed molecular characterization of PT injury, but conventional 2D cell culture systems are ineffective in modeling human PT physiology. In this study, we seek to advance the physiological relevance of *in vitro* PT models by developing and qualifying lumen-forming 3D spheroids of human cells. These self-organizing free-floating spheres demonstrate enhanced differentiated longevity and sensitivity to nephrotoxic compounds in comparison to 2D cultures. 'Proximal tubuloids' consist of a single differentiated layer of polarized cells. Primary cilia form at the apical membrane, while Na+/K+-ATPase and laminin are localized to the basolateral membrane. Using this platform, we aim to emulate *in vivo* toxicological dynamics and identify molecular perturbations that drive functional disease phenotypes. In ongoing studies, spheroids cultured in 384-well plates are exposed to longer duration repeated dosing schedules to screen for xenobiotic-induced nephrotoxicity, using clinically relevant assays and benchmark concentration analysis. Furthermore, we are extending spheroid cultures to fluidic conditions and barrier models.

24. Testing the Functionality and Reproducibility of Liver Microphysiological Systems Under Different Seeding Conditions

Alicia Lim[1], Yuki Kato[1,2], Courtney Sakolish[1], Alan Valdiviezo[1], Haley Moyer[1], Ivan Rusyn[1]

[1]Texas A&M University, College Station, USA. [2]Shionogi & Co. Ltd., Osaka, Japan

Abstract

The Texas A&M Tissue Chip Validation (TEX-VAL) Consortium is a public-private partnership that aims to promote the use of microphysiological systems (MPS), and is a collaboration of pharmaceutical and consumer goods companies, federal agencies, and a trade association with the Texas A&M University Tissue Chip Testing Center. The Consortium is engaged in stakeholder needs-directed applied research to establish the functionality, reproducibility, robustness, and reliability of a wide array of MPS. One common organ of interest to TEX-VAL stakeholders, especially for the investigation of pharmacokinetics and toxicological assessments of drugs, is the liver. Two MPS for liver studies were proposed for testing in TEX-VAL – the Mimetas 2-lane OrganoPlate® and CNBIO PhysioMimix™ MPS-LC12 plate. These models were tested for functionality and reproducibility using induced pluripotent stem cell-derived hepatocytes (iHeps) or primary human hepatocytes (PHHs) with and without non-parenchymal cells (NPCs: THP-1, HMEC-1) for up to 17 days. Imaging-based or total protein-based cell viability, albumin and urea secretion into culture media, LDH and CYP3A4 activity, and midazolam metabolism were assessed. Imaging was also used as a readout, when possible. The data collected in these studies provide important information on the utility of each model with respect to the "context of use" in which these MPS models may be applied by the end-users.

25. Assessing Drug-Induced Liver Injury Using a Sensitive and Selective Human Liver Microphysiological System and Clinical Biomarkers

Ovidiu Novac[1], Raul Silva[1], Lucy-May Young[1], Kim Lachani, David Hughes[1], Tomasz Kostrzewski[1]

[1]CN Bio Innovations Ltd, Science Park, Cambridge, United Kingdom

Drug-induced liver injury (DILI) remains the most common cause for acute liver failure in the USA and Europe and is a leading cause of attrition of compounds in drug development. As an alternative to classical 2D cell cultures, which have significant limitations in assessing DILI, we have developed a human liver microphysiological system (MPS) comprised of human primary liver hepatocyte parenchymal and non-parenchymal cells (NPCs), cultured in 3D microtissues on an engineered scaffold under perfusion up to two weeks. The methodology has been validated with a broad set of twelve severely and mildly hepatotoxic test articles with a variety of chemical composition. Liver function following drug exposure was assessed by a broad spectrum of functional liver-specific endpoints on the cellular structures and culture medium, including clinical biomarkers – alanine aminotransferase (ALT), to create a distinct mechanistic “signature of hepatotoxicity”. The MPS *in vitro* model showed superior sensitivity and specificity over classic 2D primary hepatocytes (PHHs) cultures and even some standard non-MPS 3D models in detecting DILI (sensitivity 70%, specificity 100%), and identified compounds of high clinical DILI concern that were not detected by some 2D PHHs cultures (levofloxacin) and detected mild toxicity in compounds of low-DILI concern (pioglitazone). By using a wide range of biomarkers, the liver model can detect toxicities in compounds that otherwise might be missed when using only basic cell viability endpoints. The liver MPS enables the analysis of clinical biomarkers, such as ALT, which are notoriously difficult to detect *in vitro* allowing improved translation to clinical data. Overall, we demonstrate that the liver MPS model is well suited to exploring the molecular mechanisms that underlie DILI and its association with hepatic toxicity. The model can additionally be used to assess how novel compounds behave in distinct patient subsets and how toxicity profiles may be affected by liver disease state (e.g., viral hepatitis, fatty liver disease).

AUTHOR INDEX

Author name	Affiliation	Abstract Numbers
Han, Arum	Texas A&M University, College Station, USA	20
Harrill, Alison	US EPA, Durham, NC, USA	3
Harrill, Joshua	US EPA, Durham, NC, USA	3
Hines, David	Inotiv-RTP, Morrisville, USA	16
Hoffmann, Sebastian	seh consulting + services, Paderborn, Germany	6
Hsieh, Jiu-Hua	NIEHS, RTP, NC, USA	3
Hughes, David	CN Bio Innovations Ltd, Science Park, Cambridge, United Kingdom	25
J.R. Rao, Trishaal	St. John's University, New York, USA	5
Johansson, Jenny	RISE Research Institutes of Sweden, Borås, Sweden	7
Judson, Richard	Center for Computational Toxicology and Exposure, Office of Research and Development, US Environmental Protection Agency, Research Triangle Park, NC, USA	13
Karmaus, Agnes	ILS an Inotiv Company, RTP, USA	12
Kato, Yuki	Shionogi & Co. Ltd., Osaka, Japan. Texas A&M University, College Station, USA	24
Kim, Sungjin	Texas A&M University, College Station, USA	20
Kleinstreuer, Nicole	National Institute of Environmental Health Sciences, Durham, USA	11, 12, 15, 16
Knudsen, Thomas	U.S. Environmental Protection Agency, Durham, USA	18
Kostrzewski, Tomasz	CN Bio Innovations Ltd, Science Park, Cambridge, United Kingdom	25
Kowal-Safron, Thomas	University of California, Los Angeles, USA	1
Kreutz, Anna	NIEHS, Durham, USA. ORISE, Durham, USA	14
Krishna, Shagun	National Institute of Environmental Health Sciences, Durham, USA	11
Lachani, Kim	CN Bio Innovations Ltd, Science Park, Cambridge, United Kingdom	25
Lim, Alicia	Texas A&M University, College Station, USA	24
Mansouri, Kamel	NIH/NIEHS, Durham, USA	16
Mao, Ganming	St. John's University, New York, USA	5
McFail, Alanna	Campbell University, Lillington, USA	9
Menon, Ramkumar	University of Texas Medical Branch at Galveston, Galveston, USA	20
Michelon, Camille	Predictive Biology Inc., Carlsbad, CA, USA	3
Moyer, Haley	Texas A&M University, College Station, TX, USA.	21, 24
Novac, Ovidiu	CN Bio Innovations Ltd, Science Park, Cambridge, United Kingdom	25
Nyffeler, Johanna	ORISE, Oak Ridge, TN, USA	3

Author name	Affiliation	Abstract Numbers
Oliveira, Anax	Lhasa Limited, Leeds, United Kingdom	17
Padilla, Stephanie	Center for Computational Toxicology and Exposure, Office of Research and Development, US Environmental Protection Agency, Research Triangle Park, NC, USA	13
Paul Friedman, Katie	Center for Computational Toxicology and Exposure, Office of Research and Development, US Environmental Protection Agency, Research Triangle Park, NC, USA	13
Paul-Friedman, Katie	US EPA, Durham, USA	14
Pearson, Adam	National Institute of Environmental Health Sciences (NIEHS), Durham, USA	23
Pedersen, Emma	Key2Compliance, Göteborg, Sweden	7
Pellevoisin, Christian	Urbilateria Consulting, Lyon, France.	6, 7
Phelps, Drake	North Carolina State University, Raleigh, NC, USA	2
Pierro, Jocylin	US EPA, Research Triangle Park, USA	19
Quinto, Emily	University of California, Los Angeles, USA	1
Radnaa, Enkhtuya	University of Texas Medical Branch at Galveston, Galveston, USA	20
Ramaiahgari, Sreenivasa	National Institute of Environmental Health Sciences, Durham, USA	11
Richardson, Lauren	University of Texas Medical Branch at Galveston, Galveston, USA. Texas A&M University, College Station, USA	20
Rooney, John	ILS an Inotiv Company, RTP, USA	12
Rowson, Zachary	Oak Ridge Institute for Science and Education, U.S. Department of Energy, Oak Ridge, TN, USA. Center for Computational Toxicology and Exposure, Office of Research and Development, US Environmental Protection Agency, Research Triangle Park, NC, USA	13
Rusyn, Ivan	Texas A&M University, College Station, TX, USA.	20, 21, 22, 24
Sakolish, Courtney	Texas A&M University, College Station, TX, USA.	21, 22, 24
Setzer, Rhyne Woodrow	Center for Computational Toxicology and Exposure, Office of Research and Development, US Environmental Protection Agency, Research Triangle Park, NC, USA	13
Shafer, Timothy	US EPA, Durham, USA	14
Sheats, M. Katie	North Carolina State University, Raleigh, NC, USA	2
Shipkowski, Kelly	National Institute of Environmental Health Sciences, Durham, USA	11
Silva, Raul	CN Bio Innovations Ltd, Science Park, Cambridge, United Kingdom	25
Silver, Brian	NIEHS, Durham, USA	8
Spencer, Richard	General Dynamics Information Technology, Falls Church, USA	18

Author name	Affiliation	Abstract Numbers
Stephan, Clifford	Institute of Biosciences and Technology, Texas A&M University, Houston, TX, USA	21
Tokar, Erik	NIEHS, Durham, USA. DNTP/NIEHS, DURHAM, USA	4, 8
Tu, Monika	Insphero AG, Schlieren, Switzerland	10
Valdiviezo, Alan	Texas A&M University, College Station, TX, USA.	21, 24
Vergara, Leoncio	Institute of Biosciences and Technology, Texas A&M University, Houston, TX, USA	21
Wambaugh, John	US EPA, Durham, USA	14
Wenz, Friederike	InSphero AG, Schlieren, Switzerland	10
Wetmore, Barbara	US EPA, Durham, USA	14
Willis, Clinton	US EPA, Durham, NC, USA	3
Wolf, Armin	InSphero AG, Schlieren, Switzerland	10
Wu, Xian	National Institute of Environmental Health Sciences, Durham, USA	4, 11
Yang, Xia	University of California, Los Angeles, USA	1
Yoder, Jeffrey	North Carolina State University, Raleigh, NC, USA	2
You, Dahea	NIEHS, RTP, NC, USA	3
Young, Lucy-May	CN Bio Innovations Ltd, Science Park, Cambridge, United Kingdom	25
Zamora, Zacary	University of California, Los Angeles, USA	1
Zapiorkowska, Natalia	InSphero AG, Schlieren, Switzerland	10