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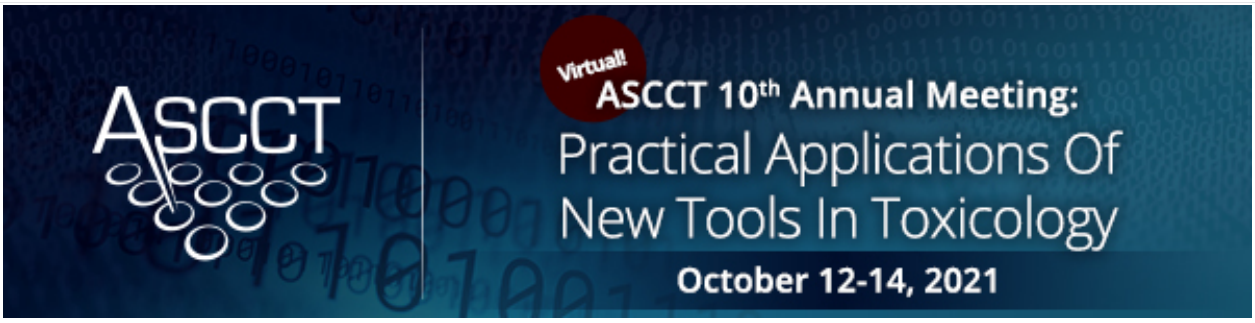

Dear Colleagues,

It is my distinct pleasure to welcome you to the 10th annual meeting of the American Society for Cellular and Computational Toxicology (ASCCT). At this major milestone, as we gather to celebrate the first decade of our society, it's appropriate to take time to reflect on where we are, from whence we came and where do we want to go. Thinking about our beginnings and progress, our successes and the lessons we've learned, as well as what have we not yet accomplished provides a basis to help us commit to continue to make an even greater difference in the development, evaluation and application of the more efficient and informative approaches to safety evaluation afforded by cellular and computational testing approaches. This type of thinking is what we tried to apply as we developed the program for this year's annual meeting and its focus on practical applications.

We hope that you too will take advantage of the opportunity the meeting offers to celebrate, reflect, and recommit. We also hope that the program content will be of interest to you, and that it will fill you with pride as you reflect what you and others have accomplished. During these jaded times might we dare to hope that it may help inspire us to continue to work collectively to make an even greater, and ever more tangible difference in the future of safety testing and evaluation for those in government agencies, industry, academia laboratories and classrooms and in the public at large both in the US and abroad?

Prudence dictates that our annual meeting be held remotely again this year thanks to Covid. Thus, we will not be able to meet and greet in person to celebrate this major milestone. However, we will celebrate remotely this year (you may wish to have a glass of your favorite adult beverage handy towards the

end of the program for day 1 for a toast) and, if all goes well, we will have a "proper" 10th year plus one in-person ASCCT celebration at next year's meeting.

In the meantime, sit back but roll up your sleeves, as we engage as a society to hear the drivers for the NAS 2007 "Toxicity Testing in the 21st Century" report, thoughts on how the promise of the vision in the report have been met, some exciting new developments, and some reflections about what needs to be done yet to have the vision fully realized. The NAS report and its adoption by EPA, FDA, NIH and beyond, as well as leadership provided by the alternatives to animal testing community, were major drivers for the establishment of the ASCCT.

Of course, we don't have time to cover all of the important topics in the field during our meeting this year, nor do we have the time and opportunity to give the topics we will address full justice, but we do hope that the talks and discussion will provide you with key highlights of what's been done and what we need to do next and beyond to realize the vision. Thank you for joining us! We hope that you will find the meeting informative and interesting. We look forward to your comments and insights as you are the society. Get ready for the program and hang tight!

John "Jack" R. Fowle III, ASCCT President

ORAL PRESENTATIONS

OR1

Plenary: Progress and Challenges of 21st Century Toxicology

Patience Browne
Organisation for Economic Cooperation and Development, Paris, France

Abstract

OECD publishes internationally harmonized test guidelines and guidance for generating chemical safety data. In addition, the programme on Good Laboratory Practices provides principles and guidance on the quality assurance environment in which the data are generated. Toxicity data that meet these two requirements are covered by the agreement on the Mutual Acceptance of Data (MAD), which mandates that these results must be accepted by all OECD member countries with a regulatory requirement for that information. MAD reduces duplicative testing, saving countries and industry an estimated € 309 million/year and thousands of animals. In addition, OECD is committed to finding alternatives to animal testing when the suitability of the alternative can be demonstrated. Molecular and cell-based methods are increasing including in OECD test guidelines to provide mechanistic information and to predict in vivo effect. In addition, uptake through the "traditional" path of including methods in test guidelines, this presentation will discuss how 21st Century Toxicity approaches have considered in projects to evaluate chemicals safety through the development of tools and guidance, and through exploratory projects to share experiences and best practices for using alternative approaches in a variety of regulatory contexts. The development of formats for reporting data are a critical piece to building structured consolidated repositories of chemicals safety information. Interoperable electronic tools can used to support meta-analyses and build predictive models. While many of the exploratory projects are not (initially) harmonized or covered by MAD, OECD projects undergo cycles of review by regulatory scientist to help define the strengths and limitation of such approaches, consider applications to regulatory decisions, and help to build confidence in using 21st century approaches for evaluating the safety of chemicals.

OR2

Screening ToxCast Chemicals in an Estrogen Receptor Transactivation Assay with Metabolic Competence

Kristen Hopperstad[1], Danica DeGroot[1,2], Todd Zurlinden[1], Russell Thomas[1], Chad Deisenroth[1]
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Abstract

The U.S. EPA continues to utilize high-throughput screening data to evaluate potential biological effects of endocrine active substances without the use of animal testing. Determining the scope and need for *in vitro* metabolism requires the generation of larger data sets that assess the impact of xenobiotic transformations on toxicity-related endpoints. The objective of the current study was to screen a set of 768 ToxCast chemicals in the VM7Luc estrogen receptor transactivation assay (ERTA) using the Alginate Immobilization of Metabolic Enzymes (AIME) hepatic metabolism method. Of the chemicals screened, 128 demonstrated estrogenic activity: 85 chemicals were positive in both assay modes (plus/minus metabolism), 16 chemicals were positive without metabolism, and 27 chemicals were positive with metabolism. To facilitate prioritization of ERTA bioactive chemicals, a novel shift metric was applied to further discriminate metabolism-dependent effects. This approach identified 88/128 active chemicals where 29 were bioactivated and 59 were bioinactivated. Human biotransformation routes and associated metabolites were predicted across the chemical library to mechanistically characterize possible transformation-related ERTA effects. Overall, the study profiled novel chemicals associated with metabolism-dependent changes in ERTA bioactivity, and suggested routes of biotransformation and putative metabolites responsible for the observed estrogenic effects. The data demonstrate a range of metabolism-dependent effects across a diverse chemical library and highlights the need to evaluate the role of intrinsic xenobiotic metabolism in endocrine and other toxicity-related health effects. *The views expressed are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA.*

OR3

Annotating and Visualizing In Vitro Data to Gain Context

Agnes Karmaus[1], John Rooney[1], Patricia Ceger[1], Jaleh Abedini[1], Jason Philipis[2], Shannon Bell[1], Dave Allen[1], Nicole Kleinstreuer[3]
[1]ILS, RTP, NC, USA. [2]Sciome LLC, RTP, NC, USA. [3]NIH/NIEHS/DNTP/NICEATM, RTP, NC, USA

Abstract

Availability of in vitro high-throughput screening (HTS) assay data is facilitating the development of computational approaches for chemical hazard assessment. Linking HTS data to regulatory endpoints remains a challenge and requires detailed information about assays as well as an understanding of biological context. For example, data from the U.S. Environmental Protection Agency's ToxCast HTS program are annotated by technology platform, assay design, and assay/gene target information, yet it remains a challenge to provide toxicological context for potential regulatory applications. Here we present a mapping approach for HTS assay endpoints that moves beyond technology-based assay annotations. Our mapping provides a robust assay grouping schema applicable beyond HTS datasets in a toxicological endpoint-based framework. This expert-led curation and annotation is available in the Integrated Chemical Environment (ICE). Annotations map assays to regulatory toxicological endpoints of interest through structured vocabularies allowing data to be searched, grouped, and visualized by regulatory endpoint. The annotations increase accessibility for those unfamiliar with individual assays by providing context for in vitro assays (and in vivo data or in silico predictions in ICE) to facilitate identification of data gaps, insight into mechanistic plausibility, and investigation into regulatory-relevant endpoints. Finally, we highlight that while single assay results are generally insufficient for regulatory application, this approach helps integrate results from multiple assays and provides data visualization to aid review of a chemical's potential activity for selected regulatory endpoints. This project was funded with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.

OR4

Patient Centric Drug Safety Assessment Evaluation of Steatosis as a Risk Factor for Drug-Induced Liver Injury

Sue Grepper
InSphero, Schlieren, Switzerland

Abstract

The sensitizing effect of steatosis and NASH as risk factors for human DILI receives a lot of attention. The reasons are the high incidence of patients with metabolic liver diseases and the need for a more differentiated patient-specific risk assessment of new drug candidates. Patients with steatosis might be more sensitive than healthy subjects. The relationship between DILI and underlying steatosis diseases has not been systematically investigated. The steatosis DILI risk factor hypothesis still needs clinical endorsement, as well supportive *in vitro* data.

Primary human hepatocytes, Kupffer and endothelial cells co-cultured as a 3D liver microtissues have been validated as a highly specific and sensitive DILI screening tool by 108 clinically tested FDA-annotated drugs (Archives of Toxicology, vol. 91, 2849-2863, 2017). Steatosis can be induced in these 3D liver microtissues within 7 days when exposed to free fatty acids and high concentrations of sugars and insulin.

The purpose of this study is to evaluate the cholestatic drug Chlorpromazine and the non-cholestatic drug Acetaminophen under steatosis and non-steatosis conditions in human 3D liver microtissues. Steatosis conditions significantly enhanced the cytotoxicity of the cholestatic drug Chlorpromazine. Conversely, steatosis did not affect the toxicity of Acetaminophen.

These preliminary results suggest that steatosis might be is a risk factor for drug-induced cholestasis. The increased cytotoxicity of cholestatic drugs might come from the intracellular accumulation of cytotoxic bile acids. Further investigations of cholestatic and non-cholestatic drugs under steatosis and non-steatosis conditions are necessary to better understand this relationship.

OR5

Characterizing intra-human variation in common toxicity endpoints in differentiated primary bronchial epithelial cell cultures.

Alysha Simmons[1], Nick Mallek[1], Emily Aungst[2], Shaun D. McCullough[2]
[1]UNC-Chapel Hill, Chapel Hill, NC, USA. [2]US EPA, Chapel Hill, NC, USA

Abstract

The use of primary human bronchial epithelial cells (pHBEC) has increased in recent years because they can be differentiated under air-liquid interface (ALI) culture conditions and offer greater *in vivo* physiological relevance than their cell line counterparts. pHBEC ALI models may also provide insight into the intra-human (i.e., within the human population) variation in the response to test agents that cannot be addressed by isogenic cell lines or inbred rodent strains. These models also provide the opportunity to incorporate complex concepts such as susceptibility into *in vitro* testing approaches. One fundamental challenge facing the interpretation of toxicity data from these models is a lack of consensus on the normal range of variation in commonly used endpoints and definition of the magnitude of an exposure-induced change required for an effect to be considered pathologic. Here we sought to characterize the pre- and post-exposure ranges of variability for culture morphology, trans-epithelial electrical resistance (TEER), ciliary beat frequency (CBF), and pro-inflammatory gene expression in organotypic *in vitro* bronchial tissues constructed from donor-matched, co-cultured pHBEC and human lung fibroblasts. Preliminary experiments revealed that co-cultures eliminated the formation of cyst-like structures in the epithelium and promoted functional and molecular divergence that epithelial mono-cultures did not recapitulate. Co-cultured pHBECs exhibited similar cell-type diversity, CBF, and viability characteristics across donors; however, donors exhibited distinct mature TEER values and pro-inflammatory gene expression signatures after exposure to environmentally-relevant concentrations of acrolein and ozone (i.e. *COX2, IL8, IL6*). Future work will characterize and identify applications of this model. This work does not reflect EPA policy.

OR6

Plenary: Transcriptomics in Human Health Risk Assessment: Lessons From Canadian Research-Regulatory Collaborations

Carole Yauk
University of Ottawa, Ottawa, Ontario, Canada

Abstract

Regulatory toxicology testing is undergoing modernization to decrease animal testing and increase efficiencies and human predictivity. At the center of these efforts is establishing acceptable approaches for the use of transcriptomics to advance regulatory testing strategies. Laboratories are now pairing RNA-sequencing and high-throughput transcriptional profiling with powerful bioinformatic pipelines to provide an unprecedented amount of toxicological information to inform the molecular alterations induced by chemical exposures. This presentation will describe research-regulatory collaborations undertaken within Canada and with international partners to advance these applications. Specifically, our research has worked to: (1) develop and apply transcriptomic biomarkers and gene set enrichment analyses for hazard and mode of action identification; and (2) apply transcriptomic benchmark dose (BMD) analysis to estimate points of departure. We are applying these tools in case studies with regulatory partners, which are critical for defining context of use and advancing new approach methodologies. These case studies include analyses of single chemicals and small chemical groups, and span chemical prioritization, chemical grouping, potency comparison, tiered-testing, and derivation of a transcriptomic point of departure for use in risk assessment. Exemplary case studies will be shown on lessons learned from case studies on the flame retardant hexabromocyclododecane (tiered testing approach), and a set of per- and poly-fluorinated substnaces in which transcriptomics was used to identify chemical similarities, characterize relative potency, and derive bioactivity exposure ratios. Use of the Organisation for Economic Co-operation and Development Transcriptomic Reporting Framework and the Regulatory Omics Data Analysis Framework (ODAF) to facilitate regulatory submissions will be discussed. These case studies provide a context for understanding the applicability and implications of using transcriptomics for risk assessment.

OR7

Development of a novel genotoxicity prediction model based on biomarker genes in human HepaRG™ cells

Anouck Thienpont[1,2], Stefaan Verhulst[1], Leo van Grunsven[1], Vera Rogiers[1], Tamara Vanhaecke[1], Birgit Mertens[2,3]
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[3]Universiteit Antwerpen, Antwerpen, Belgium

Abstract

To improve the predictive capacity of genotoxicity testing, retrieving mechanistic information from relevant human cell systems is crucial. This can be achieved *via* new approach methodologies (NAMs) which include gene expression biomarkers. We previously developed the gene signature GENOMARK, consisting of 84 biomarker genes to identify genotoxic substances in metabolically competent human HepaRG™ cells. GENOMARK showed an accuracy of 100% using a reference dataset of 24 chemicals and a support vector machine (SVM) model for classification. In the present study, a further optimized prediction model was developed based on an extended reference dataset of 38 chemicals. To this extent, new test data was generated and the performance of two supervised machine learning algorithms in R software, i.e. SVM and random forest (RF), was compared based on the extended dataset. Both prediction models showed the same predictive accuracy (92.3%), although the RF model displayed a higher sensitivity and a lower specificity compared to the SVM model. Moreover, the RF model was less sensitive to outliers for 1 or more biomarker genes. Both models were applied to predict the genotoxicity of 6 misleading false positive chemicals (2-methyl-4-isothiazolin-3-one, 4-amino-3-nitrophenol, sodium benzoate, dihydroxyacetone, hydroxybenzomorpholine and 1-napthol). Overall, the RF model appeared to be the most appropriate to classify chemicals for genotoxicity based on the 84 biomarker genes. These results demonstrate that combining gene expression data with supervised machine learning algorithms can play an important role in the ongoing paradigm shift in genotoxicity toxicology to move towards a more human-relevant genotoxicity testing.

OR8

Best Practices for Benchmark Dose Analysis of Toxicogenomic Data

Ruchir Shah[1], Michele Balik-Meisner[1], Deepak Mav[1], Dhiral Phadke[1], Jason Phillips[1], Alex Sedykh[1], Will Gwinn[2], Scott Auerbach[2]
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Abstract

Toxicogenomic benchmark dose (BMD) analysis addresses an increasing need of the scientific community to utilize genomic dose response data in toxicology, drug design, risk assessment, and translational research, with an important goal of providing an estimate of the level of chemical exposure at which a biological response is initiated (the BMD). Different aspects of a study design such as dosing, sample size, and signal strength, create challenges for identifying appropriate thresholds for underlying parameters and criterion. In turn, the options effect the ability of the models to accurately estimate gene- and pathway-level BMDs with certainty. Additionally, the current BMD analysis recommendations are not developed specifically for transcriptomic data types. To address these study design and analyses questions, we have developed two types of datasets: 1) "null or control" datasets simulated through random mixing of control/unexposed samples from actual exposure studies, and 2) dose responsive datasets with varying degrees of spiked in dose response effect for varying numbers of genes. The simulated null datasets are utilized to explore false positive discovery rate, and the simulated dose responsive datasets are implemented to assess, and balance, true positive discovery rates across the same parameter or design options. We discuss findings from extensive toxicogenomic dose response analysis using BMDExpress2.3 and rigorous statistical analysis of simulated datasets to share our findings on analysis choices. We specifically include evaluation of data logging, comparison of types of confidence interval calculations deployed for BMDs, prefiltering methods, and additional parameter and design considerations for toxicogenomic dose response analysis.

OR9

Plenary

Thomas Hartung
Johns Hopkins University, Baltimore, USA

OR10

Plenary: Advances in Applying Machine Learning in Toxicology

Sean Ekins
Collaborations Pharmaceuticals, Raleigh, USA

Abstract

Computational toxicology covers a number of different approaches using rules, models and algorithms to predict discrete molecular endpoints or broader effects. The applications are similarly broad from assisting in prioritizing molecules for drug discovery, scoring components in consumer products and assessing molecules effects on the environment, as just a few. Our focus is predominantly on ligand-based approaches such as machine learning (ML) to show how these models are useful for industry (pharmaceutical, consumer products and regulatory applications). Over the last few years, we have compared various ML methods and performed external testing for endocrine disruption models (estrogen receptor, androgen receptor and aromatase models). We have also developed and validated several ML models for drug induced liver injury (DILI) which can be used to predict whether drugs and other molecules may have this toxicity. Such models are generally on a par with in vitro data being generated in hepatocytes and 3D cultures. These are a small selection of the hundreds of ML models we have developed as part of our MegaTox^{Ò} platform. Some of the ML approaches are computationally expensive with very large datasets so we have assessed quantum machine learning on a quantum computer (QC) as a potential approach to accelerate model building. If QC becomes commercially viable these may have utility for computational toxicology in future. These and our latest developments will be described as we expand the applications of ML with new technologies and datasets.

OR11

Machine learning pipelines to support automation of literature systematic methods for the development of alternative toxicological methods

Kristan Markey[1], Kellie Fay[1], Andrea Kirk[2], Scott Lynn[1], Andy Shapiro[3], Sara Vliet[4], Peter Baumgartner[5], Robert Chew[5], David Henderson[5], Anna Adetona[6], Matthew Austin[6], Po-Hsu Chen[6], Mitch Gauthier[6], Joeseph Kay[6], Mark Lancaster[6], Jordan Vasko[6], Amy Thomas[6], Stephen Edwards[5], Michelle Angrish[3]
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Abstract

This aim of this work is to reduce the effort needed to identify and extract information from scientific literature in support of systematic approaches for the environmental health and safety and new approach methodologies (NAMs) domains, for example, to support reference chemical identification for validation of NAMs. To that end, we are developing automated solutions across the systematic method pipeline.

To improve literature searches, we are deploying query expansion technologies using community-developed lexical resources such as the Unified Medical Language System. These tools extend expert-generated search concepts by 100s of related keywords identifying 1000s of additional articles but require pipelines to target results to a manageable scale. For a cross-species androgen receptor in vitro example, total articles increased >10x while relevant articles increased by 3.5x. Numerous approaches including clustering, ontological annotations, weak supervision, and deep learning models on titles and abstracts are then used to prioritize the articles for review. Manual QC efforts, by using ontological annotations with support vector machine learning, for example, are reduced by 20-40% compared to the traditional systematic review 2-screener approach.

We are also developing tools to facilitate automated labeling and extraction from full-text studies. The data are mapped into common fields based on lexical resources to facilitate rapid identification and integration.

We discuss the challenges, performance, and opportunities of the tools for our domains and others.

The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the US EPA Environmental Protection Agency.

OR12

Towards Automating Information Extraction with FIDDLE: From Text Annotation to Interoperable Information Extraction via Machine Learning

Brian Howard, Arpit Tandon, Christopher Norman, Tyler Albert, Shyam Patel, Rebecca Elmore, Lena Schmidt, Ruchir Shah
Sciome LLC, Research Triangle Park, NC, USA

Abstract

Systematic review has recently gained significant popularity in several disciplines including environmental health and evidence-based toxicology. we are conducting research and development of a semi-automated data extraction platform for use in this context. First, we are currently working on the "software 2.0" version of our PDF text extraction software which utilizes deep learning, image processing, and NLP to convert binary PDF text documents into machine-readable raw text. Second, we have developed a web-based platform designed to allow users to efficiently annotate text with entities, groups and relations that are of interest for a given data domain. Finally, we are using the resulting datasets to build high-quality neural machine learning models for automated information extraction and normalization.

Because accurate data extraction can be a challenging problem, and given that current methods rarely achieve 100% accuracy, all of the resulting methods will be integrated into a "human-in-the-loop" system that combines machine and human intelligence in a manner that is superior to using either in isolation. The system will: highlight extracted terms in a pdf; automatically populate extraction forms with extracted data; allow humans to intervene and correct the results; and learn from the corrections to continually update the model. Furthermore, by defining and supporting standardized interfaces for various information processing tasks, our system is designed to facilitate the incorporation of extraction components developed by external providers and academic research groups. Our overarching goal is to translate emerging semi-automated extraction technologies out of the lab and into practical software.

OR13

A machine learning-based predictive model of ligand-induced genome-wide binding of the aryl hydrocarbon receptor

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Abstract

The Aryl Hydrocarbon Receptor (AhR) is an inducible transcription factor (TF) whose ligands include the potent environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). TCDD-mediated toxicity is believed to occur through activation of AhR and its binding to the 5'-GCGTG-3' DNA motif, referred to as the Dioxin Response Element (DRE). However, AhR binding in intact human cells is highly dynamic and tissue-specific. Approximately 50% of all experimentally verified AhR binding sites do not contain a DRE, and a great number of otherwise accessible DREs are not bound by AhR. Identification of the determinants of tissue-specific AhR binding is crucial for understanding downstream gene regulatory effects and potential adverse health outcomes of TCDD exposure, such as liver toxicity and immune suppression. We applied XGBoost, a supervised machine learning architecture, to predict genome-wide AhR binding as a function of DNA sequences immediately flanking the DRE, and local chromatin context features such as DNase-seq, histone modifications, and TF ChIP-seq signals, as well as DRE proximity to gene promoters. We predicted binding of induced AhR in MCF-7 breast cancer cells, human hepatocytes, and the human lymphoblastoid cell line GM17212, as well as constitutive AhR binding in HepG2 cells. Our results demonstrate highly accurate and robust models of within-tissue binding, with several specific TFs and HMs identified as predictive of AhR binding within and across tissues. Additionally, we show that tissue-specific AhR binding is driven by a complex interplay of DNA flanking sequence and local chromatin context.

OR14

Plenary: Thirty Years in the Validation of Computational Models – Lessons Learned and Challenges Ahead

Andrew Worth
Joint Research Centre, Ispra, Italy

Abstract

This presentation will discuss the use of computational models in the regulatory assessment of chemicals, focussing on the role of validation in establishing model

credibility and thus acceptable use. Computational models will be compared and contrasted with experimental (in vitro) methods in terms of the principles and procedures of validation. Over the past 30 years, different validation frameworks have evolved somewhat independently for different methodologies, including Quantitative Structure-Activity Relationship (QSAR) models, read-across approaches, and physiologically based toxicokinetic models. In comparing these frameworks, an important question is whether the differences are more apparent than real. This is not merely an academic question, but one of practical significance, since chemical safety assessments are increasingly dependent on the need to integrate multiple and diverse data streams. When combining multiple methods within Integrated Approaches to Testing and Assessment (IATA), there is also a need to combine their uncertainties. This presentation will reflect on current validation practices, and consider the extent to which they serve the chemicals policy goals of the 21st century.

OR15

Plenary: Good in Vitro Method Practices and Validation Capacity Building for Accelerating Toxicological Decision-Making Based on Human Relevant Mechanistic Methods

Sandra Coecke
Joint Research Centre, Ispra, Italy

Abstract

The first guidance on Good Cell Culture Practice (GCCP) dates back to 2005 (Coecke et al. 2005). With the availability of more complex cell and tissue culture system like human induced pluripotent stem cells or organ-on-chip devices with microfluidic technologies, the potential applications of human cell culture models has been greatly broadened. When using the next generation culture models in research, product development, testing and manufacture of biotechnology products and cell-based medicines, it remains critical to include aspects of quality assurance. As such, the original set of GCCP principles of best practice can be used as a basis to assure good cell and tissue practices and conditions when working with cell culture systems in simple set-ups or in very technological advanced formats (Pamies et al., 2017). Applying GCCP as part of overall Good In Vitro

Method Practices (GIVIMP, OECD 2018) by the global life science community is leading to more harmonisation of in vitro method related processes and procedures. Researchers are key players to ensure use of such best scientific and quality practices and are ideal ambassadors to use them in the novel generation of stem cell and tissue–based methods. The European Commission Joint Research Centre's European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) is working on developing and validating innovative, mechanistic methods and approaches based on current harmonized scientific and quality standards complying with the GCCP principles and the GIVIMP guidance document (OECD, 2018) and is crowd-sourcing for human relevant mechanistic methods into the required regulatory test batteries for identifying thyroid disrupting chemicals using the European Union Network of Laboratories for the Validation of Alternative Methods (EU-NETVAL) validation capacity. Key instruments to disseminate globally harmonized good cell, tissue and method practices are published principles and guidance, conferences, face-to-face meetings, training, webinars, templates (Krebs et al., 2019), reporting and evaluation tools (e.g. http://www.scirap.org/) and e-learning training materials.

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OR16

Establishing Confidence in NAMs: Considering Variability in Reference Test Methods

David Allen[1], John Rooney[1], Neepa Choksi[1], Patricia Ceger[1], Amber Daniel[1], Agnes Karmaus[1], Judy Strickland[1], Kim To[1], Jim Truax[1], Warren Casey[2], Nicole Kleinstreuer[2]
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Abstract

Many hazard classification and labeling systems are based on in vivo test method results. In vivo methods are also typically used as the benchmark against which new approach methodologies (NAMs) are compared. For many toxicity endpoints, there is no NAM accepted as a complete replacement of animal use due to a lack of concordance with the full spectrum of hazard categories. However, does discordance with in vivo results always indicate that the NAM is “wrong”? Variability of results from in vivo test methods could be an important contributor to such discordance and therefore should be carefully considered when comparing in vivo and NAM results. It is critical to understand any variability inherent to the animal test, as this variability will directly affect the expectations for performance of NAMs that seek to replace it. Sources of such variability might include both the inherent variability among animals and the subjective nature of observational in vivo endpoints. This presentation will summarize efforts at

NICEATM to characterize the variability of in vivo reference test methods for multiple endpoints, including skin and eye irritation, skin sensitization, and acute systemic toxicity. For example, we analyzed in vivo skin irritation data and found that chemicals classified as mild irritants were less than 50% likely to be repeated as such if retested. These efforts provide the basis for benchmarks against which to evaluate NAMs, and thereby set appropriate expectations for NAM performance. This project was funded with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.

OR17

Use of cause-and-effect analysis to optimize the reliability of in vitro inhalation toxicity measurements using an air-liquid interface

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Abstract

In vitro inhalation toxicology methods are increasingly being used. Although the opportunity for increased human relevance of in vitro inhalation methods compared to in vivo tests has been established and discussed, how to systematically account for variability and maximize the reliability of these in vitro methods, especially for assays that use cells cultured at an air liquid interface (ALI), has received less attention. One tool that has been used to evaluate the robustness of in vitro test methods is cause-and-effect (C&E) analysis, a conceptual approach to analyze key sources of potential variability in a test method. These sources of variability can then be evaluated using robustness testing and potentially incorporated into in-process control measurements in the assay protocol. There are many differences among in vitro inhalation test methods including the use of different types of biological test systems, exposure platforms/conditions, substances tested, and endpoints, which represent a major challenge for use in regulatory testing. In this manuscript, we describe how C&E

analysis can be applied using a modular approach based on the idea that shared components of different test methods (e.g., the same exposure system is used) have similar sources of variability even though other components may differ. C&E analyses of different in vitro inhalation methods revealed a common set of recommended exposure system and biological in-process control measurements. The approach described in this paper should help improve the inter- and intralaboratory agreement of in vitro inhalation test results, leading to increased confidence in these methods.

OR18

Acute Oral Toxicity Predictions for Environmental Risk Assessment

Kamel Mansouri[1], Nicole Kleinstreuer[1], Warren M. Casey[1], David Allen[2], Patricia Bishop[3]
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Abstract

To address the pressing need to rapidly and accurately assess the safety of environmental chemicals and reducing the number of animals used in regulatory testing while still protecting wildlife, NICEATM and the ICCVAM Acute Toxicity Workgroup organized a global collaborative project to develop predictive *in silico* models of acute oral systemic toxicity potential. Participants from 35 international groups submitted a total of 139 models built using a dataset of 11,992 chemicals split into training (75%) and evaluation (25%) sets. These crowdsourced models were developed for five endpoints identified as relevant to regulatory decision frameworks: LD_{50} value, EPA hazard categories, GHS hazard categories, very toxic (LD_{50} < 50 mg/kg), and non-toxic (LD_{50} > 2000 mg/kg). Predictions within the applicability domains of the submitted models were evaluated and combined into consensus predictions based on a weight-of-evidence approach. The resulting Collaborative Acute Toxicity Modeling Suite (CATMoS), leverages the strengths and overcomes the limitations of individual modeling approaches. The consensus model predictions are fully reproducible and demonstrated equivalent performance to in vivo data offering a strong potential replacement for animal testing. Based on these results, CATMoS predictions for selected chemicals are

currently being evaluated in comparison to rat LD_{50} tests from publicly available ecological risk assessments. *This abstract does not necessarily reflect NIEHS policy.*

OR19

Identifying the Molecular Mechanisms of Air Pollution-Induced Thrombosis

Eva Vitucci[1], Shaun D. McCullough[2]
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Abstract

Approximately 3.5-million people die annually from air pollution-induced cardiovascular disease (API-CVD). API-thrombosis (API-T) is a main contributor of these mortalities; however, the molecular mechanisms driving API-T are unclear. To identify these mechanisms, we developed a tri-culture in vitro model that represents the interface of the respiratory and cardiovascular system, the alveolar capillary region (ACR). This organotypic model includes human alveolar-like epithelial cells (H441), human lung fibroblasts, and human lung microvascular endothelial cells (HULEC). We hypothesized that air pollutant exposure of the H441 would initiate the onset of a pro-thrombotic state in the HULEC. To test this, we exposed H441 to the ubiquitous air pollutant, diesel exhaust particulates (DEP), and investigated the effect of this trans-alveolar exposure (TA-DEP) on the underlying HULEC. TA-DEP exposure reduced glutathione redox potential and induced expression of anti-oxidants such as HMOX-1. Concurrently, TA-DEP decreased expression of the endothelial anti-coagulant genes, TPA, TPU, and THBD, and increased expression of the procoagulant gene, F3. TA-DEP exposure also increased NRF2 protein and activation of ERK1/2. Pharmacologic inhibition of ERK1/2 activation in the HULEC had no effect on the above TA-DEP gene expression changes, yet pharmacological inhibition of ERK1/2 activation in the H441 prevents the significant TA-DEP induced gene expression changes in the HULEC. These data suggest that TA-DEP exposure induces redox dysfunction and an endothelial pro-thrombotic profile in the ACR that may be driven by secreted epithelial mediators. We conclude that redox dysfunction and pro-thrombotic activation in the capillary beds of the ACR may be critical initiation steps of API-T. Does not reflect EPA policy.

OR20

New In Vitro Platform for High Throughput Screening of Neurons Enables Automation of Neurotoxicity Assays

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Abstract

Numerous compounds have been associated with alterations in neurodevelopment including medications, cosmetics, and pesticides. Animal experiments with rats are currently the gold standard in developmental neurotoxicity testing. However, it is not feasible to test all compounds in the market with current guidelines due to high costs; long testing times, high number of animals required and low reproducibility. We have developed technology to rapidly grow neurons-on-a-chip, precisely organizing neuronal networks at the single cell level for rapid evaluation of changes in neuronal morphology and connectivity (Magdesian et al., Biophys J. 2016; JOVE 2017). We combined these technologies in multi-well microplates to reproducibly test over 3,000 neurons per plate in 30 min. using standard plate readers. NeuroHTS™ microplates can rapidly evaluate the effects of thousands of compounds on neuronal morphology, neurite growth, synapse, and network formation with over 85% plate to plate reproducibility. Here, we exposed human neurons grown in NeuroHTS™ to different concentrations of mercury and we developed a software application to automate the measurement of neurite outgrowth, neuronal and oligodendrocyte differentiation. Compared with current commercial platforms, our results are obtained at least 10x faster and with higher precision and accuracy. The NeuroHTS™ is the first multi-well microplate to enable testing of compound toxicity just to axons or to whole neurons, resulting in robust analysis of neuronal morphology in HT. The main advantages of our technology are faster acquisition of neuronal data and generation of more predictive data of compounds' safety and efficacy prior to exposure to humans.

OR21

Development of *in vitro* human alveolar tissue models for SARS-CoV-2 Research using novel electrospun scaffolds

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Abstract

In vitro models of human alveolar air-blood barrier (ABB) tissues are needed for COVID-19 research and therapeutics development. However, high throughput (HTP) formats of these tissue models are lacking. We are developing HTP human ABB tissue models by adapting novel electrospun scaffolds to HTS Transwell®-24 and 96 well permeable support plates (Corning Life Sciences). BioSpun™ biodegradable poly(lactic-co-glycolic acid) (PLGA) scaffolds were prepared using solution electrospinning and post-treatment processes. Human pulmonary microvascular endothelial cells (HPMEC) were seeded onto the underside (blood-side) of the electrospun scaffold, and normal human alveolar epithelial cells (HAEpiC) were seeded onto the top (air-side). Scaffolds were cultured at the air-liquid interface to produce ABB tissue models. The thin electrospun scaffolds, which are biodegradable, diminished with time to allow closer, more physiologic interaction between the endothelial and epithelial cells compared to previously described *in vitro* ABB models. Transepithelial electrical resistance (TEER) measurements, utilized to determine the barrier properties of the ABB models, showed stable, long-term TEER values as high as 2,300 $\Omega \times cm^2$, demonstrating extremely robust epithelial barrier. Immunohistochemical (IHC) staining demonstrated expression of SARS-CoV-2 receptors and cell specific markers. A SARS-CoV-2 pseudovirus expressing the SARS-CoV-2 spike protein was shown to bind to both the epithelial and endothelial components of the models. These results indicate that HTP *in vitro* human ABB models produced on novel electrospun scaffolds are unique and useful models for SARS-CoV-2/COVID-19 research and will likely find utility for wider applications in respiratory infection, toxicology and drug delivery as well.

OR22

Utilizing ICE Tools to Expand Chemical Knowledge: Chemical Quest

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Abstract

The National Toxicology Program's Integrated Chemical Environment (ICE) provides easy access to data and tools to explore and contextualize chemical bioactivity. The interactive computational tools in ICE allow users to characterize, analyze, and predict bioactivity for their chemicals of interest. ICE Search provides summary-level information, curated reference data, and bioactivity details for chemicals and mixtures. However, developing an overall picture of potential bioactivity for a chemical can be a difficult challenge if there are limited data available for the chemical. ICE Chemical Quest allows users to explore ICE's database of over 800,000 chemicals through SMILES or 2D renderings, providing information on target chemicals and those with similar structures. Query results are ranked based on the similarity of the fingerprints of the query chemicals with chemicals in the ICE database. ICE returns drawings of all returned structures so the user can evaluate the appropriateness of the output list. These similar chemicals can then be entered into any ICE tool, including the Physiologically Based Pharmacokinetics and In Vitro to In Vivo Extrapolation tools, expanding available information and enhancing queries. This presentation includes case studies to provide an overview of the resources available in ICE for chemical analyses and comparisons. This project was funded with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.

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- Discrete and targeted introductions between industry, CRO and technology providers
- Decades of experience in and connections within pharmaceutical, crop protection, cosmetic, household product and chemicals industries

Poster P068: The terms "alternatives" and "alternatives to animals" should be confined to the history books.

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POSTER PRESENTATIONS

Poster Presentations

PO1

Quantitative Structure-Activity Relationship (QSAR) Modeling to Predict the Transfer of Environmental Chemicals across the Placenta

Laura Leveque[1,2], Nadia Tahiri[1,2], Michael-Rock Goldsmith[3], Marc-André Verner[1,2]
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Abstract

The increasing diversity of environmental chemicals in the environment, some of which may be developmental toxicants, is a public health concern. The aim of this work was to contribute to the development of rapid and effective methods to assess prenatal exposure. Quantitative structure-activity relationships (QSAR) modeling has emerged as a promising method in the development of a predictive model for the placental transfer of contaminants. Fetal to maternal plasma or serum concentration ratios for 105 chemicals were extracted from the literature, and 214 molecular descriptors were generated for each of these chemicals. Ten predictive models were built using Molecular Operating Environment (MOE) software, and the Python and R programming languages. Training and test datasets were used, respectively, to build and validate the models. The Applicability Domain Tool v1.0 was used to determine the applicability domain. The models developed with the partial least squares regression method in MOE and SuperLearner in R, showed the best precision and predictivity, with internal coefficients of determination (R2) of 0.88 and 0.82, cross-validated R2s of 0.72 and 0.57, and external R2s of 0.73 and 0.74, respectively. The inclusion of all test chemicals by the domain of applicability demonstrated the reliability and relevance of the model predictions. The results obtained demonstrate that QSAR modeling can help quantify placental transfer of environmental chemicals.

PO2

Using imputation to predict in vitro toxicity

Moritz Walter[1], Luke Allen[1], Antonio de la Vega de León[1], Samuel J. Webb[2], Valerie J. Gillet[1]
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Abstract

Multi-target in vitro toxicity datasets such as Tox21 and ToxCast are potentially useful to better understand and predict human toxicity. However, multi-target toxicity datasets are typically sparse, i.e., not every compound was tested in every toxicity assay. Imputation describes the process of predicting missing values in a sparse dataset and may therefore be a useful way of filling crucial gaps in knowledge. In contrast to classical QSAR models, which are based solely on relations between chemical descriptors and toxicities, imputation models leverage relations between different assays to make predictions.

In the present study, two different imputation approaches were compared to classical QSAR models using various multi-target toxicity datasets including the ISSSTY dataset (Ames mutagenicity in different bacteria strains) and the high-throughput toxicity screening datasets Tox21 and ToxCast. The imputation approaches are Feature Nets, which uses related assays as additional descriptors in supervised machine learning models, and Macau, a matrix factorization technique. Both imputation approaches clearly outperformed classical QSAR models. Further analyses revealed that the effectiveness of imputation models increased with the amount of available training labels and that benefits compared to standard QSAR models are particularly strong for compounds that are dissimilar to compounds in the training set. Hence, imputation models may possess a wider applicability domain compared to classical QSAR models.

Our results show that imputation represents an attractive approach to predict toxicity in sparse datasets. The benefit over classical QSAR models is achieved by incorporating experimentally determined labels of related toxicity assays into the models.

PO3

Specific pesticides associated with parkinsonism: A systematic review and meta-analysis

XI CHEN, Jung-San Chang, Hung-Lin Kan, Ying-Chi Lin
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Abstract

Pesticide exposure had been considered as a major environmental risk factor for Parkinson's disease (PD). Despite of structure diversity, pesticides were seldom evaluated individually. The purpose of this study was to assess the association between specific pesticides and PD through systematic review and meta-analysis. Names of insecticides, fungicides, herbicides, fumigants and terms related to PD were used as the key words for PubMed and Embase search. No language restrictions were set. Cohort or case-control studies comparing PD incidence between specific pesticides and non-exposure group were included for data extraction. Study quality was assessed by Newcastle-Ottawa Scale. Meta-analysis was performed by Revman 5. Ten studies, including 2 cohort studies and 8 case-control studies, with median to high study quality and fulfilled our pre-determined inclusion and exclusion criteria were analyzed. Eighteen specific pesticides can be evaluated. Eight pesticides, namely aldrin, dieldrin, malathion, parathion, chlorpyrifos, phorate, permethrin, and 2,4-D, were identified as significantly associated with PD. Interestingly, paraquat and maneb, two pesticides commonly used for PD animal models, were not significantly associated with PD. More studies on the mechanisms of the pesticides to elucidate their association with PD will be warranted.

PO4

Data Annotation and Migration Across Systematic Review Tools

Michelle Angrish[1], Parnian Soleymani[2], Brian Howard[3], Andy Shapiro[1], Derek Lord[4], Michele Taylor[1]
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Abstract

Automatic update of the information supporting an assessment is limited by manual finding, uploading, and migration of information between various systematic review tools. This is a particular challenge in the development of chemical assessments where a rapid compilation of new information and/or update to existing information is needed in a pre-decisional, regulatory context. Artificial intelligence was previously used to rapidly screen >40K studies for ~150 perfluoroalkyl substances and manually extracted data were summarized in systematic evidence maps (SEMs). Keeping these influential SEMs updated with the latest relevant research is time-consuming and labor-intensive. Our goal is to use machine learning models to reduce this ongoing effort. However, creating such models first requires detailed, machine-readable annotation of datasets to label entities of interest. The structured data extraction templates supporting DistillerSR for the PFAS SEM were used to create entities (labels for text) that were manually annotated from the titles, abstracts, methods, and results sections from 67 animal toxicology PDFs using the FIDDLE Extraction Workbench. A total of >24K annotations were generated from 12 entities across the corpus. Pilots targeting the migration of annotated data outputs revealed the needs that are the focus of current work. These include mapping annotated terms that do not follow convention (e.g. linking to controlled vocabularies and ontologies),piloting annotation tool grouping and relating annotations according to predefined schema, and input/output formats that support interoperability between tools. These views do not necessarily reflect those of the US EPA.

PO5

Advancement in quality of Cosmetic Product Information File by *In-silico* approach

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Abstract

Today, globally there is a regulatory obligation to stop animal testing and switch to alternatives methods. Toxicity Testing in 21st century program was initiated. This approach was considered as Next Generation Risk Assessment (NGRA) that offers a suitable framework for safety assessment. NGRA makes use of animal-free New Approach Methodologies (NAMs). NAMs consist of *in-chemico*, *in-silico*,

in-vitro and *ex-vivo* methods. Cosmetics and personal care products are the first categories to decommission animal studies. Many alternatives are currently being used to furnish a safety report called Product Information File (PIF). In a PIF safety of INCIs of a cosmetic is evaluated. Under extreme situations in case of data gaps, TTC is being used. A more refined approach is the application of iTTC. However, due to time constraints or inaccessibility of published literature, the toxicologist is unable to review full literature, and many times POD/[L]NO[A]EL misses out. This data gap is either filled with TTC and/or assessed basis of phy-chem parameters. This approch causes unnecessary conservativeness for setting MoS for an INCI. To overcome this issue, we integrated AI and computer language-based Data mining and Text scripting in our R&D to fetch out that available but inaccessible data. A pilot test was run to verify it and we were successful. We are happy to share the insights and believe that our approach will reduce the time and cost of drafting efficient and detailed CPSR for safety evaluation; with data backed up using *in-silico* tools too, on a case-by-case basis.

PO6

An In Silico Workflow for Translational Safety Assessment Illustrated using Drug-Induced Liver Injury

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Abstract

Translational safety assessment has recently become the focal point of many computational approaches. Although many publications describe the use of QSAR modeling, genomics, and artificial intelligence, practical approaches are still needed to integrate and aggregate such diverse information. Using drug-induced liver injury (DILI) as an example, in this study we present an in silico workflow for utilizing both preclinical and clinical information. DILI is one of the most difficult endpoints in drug discovery as well as in chemical safety assessment, as is relating preclinical animal data to human/clinical findings. Our proposed workflow successfully leverages existing preclinical and clinical data along with mechanistic and metabolism knowledge to better translate mammalian to human DILI. For a

given query molecule, both human and mammalian models are used to generate predictions. If the predictions conflict, both metabolites, and parents are investigated for structure−activity relationships and species-differentiating chemotypes. The results of the models are combined using Dempster−Shafer decision theory to yield a final outcome for human DILI including the estimated uncertainty. Finally, these tools are implemented within an in silico platform for systematic evaluation and use case examples are presented. This approach can be extended to include other types of new approach methodologies (NAM) data for translational chemical safety assessment.

PO7

Incorporation of Historical Control Data in the Detection of Treatment-Related Effects in Toxicology Studies via Bayesian Inference

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Abstract

Detection of treatment-related effects in nonclinical *in vivo* toxicology studies can be difficult as these studies are typically underpowered to statistically evaluate the wide range of endpoints assessed. Subjective heuristic approaches are commonly employed to adjudicate whether or not a true treatment-related effect was observed by comparing the magnitude of a potential treatment effect against the historical distribution of the observed endpoint among control animals; however, relevant historical control data are often not readily available for actionable use. Fortunately, electronic standardized CDISC-SEND-formatted datasets, now routinely generated along with toxicology study reports submitted to the Center for Drug Evaluation and Research (CDER) at FDA, can function as a repository of historical control data, enabling the use of Bayesian inference to formally incorporate historical data into the interpretation of current study data. R scripts were written to extract relevant historical control data from a repository of SEND datasets and apply Bayesian inference to calculate the posterior probability of a treatment effect, given the data observed in the study of interest and the prior, i.e., the historical distribution of the study endpoint. These scripts were developed into a user-friendly R Shiny web application. The posterior

probabilities reported by the application provide useful estimation as to the likelihood that an observed potential treatment effect is real by formally accounting for the background distribution of the study endpoint in similar studies that were previously submitted, according to the criteria set by the user, e.g. animal species, age, route of administration, etc.

PO8

QSAR models Supporting Toxicokinetic Modeling and In Vitro to In Vivo Extrapolation

Kamel Mansouri[1], Xiaoqing Chang[2], David Allen[2], Warren Casey[1], Nicole Kleinstreuer[1]
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Abstract

Physicochemical properties affecting absorption, distribution, metabolism, excretion are of high importance to physiologically based pharmacokinetic (PBPK) and toxicokinetic (PBTK) modeling as well as in vitro to in vivo extrapolation (IVIVE) studies. OPERA was developed to offer a free and open-source/open-data suite of QSAR models for endpoints including plasma protein binding (Fu), intrinsic hepatic clearance (Clint), acidic dissociation (pKa), octanol-water partition and distribution coefficients (logKow, logD) in addition to other physicochemical and environmental fate properties. All OPERA models are built on curated data and standardized QSAR-ready chemical structures. OPERA follows the five OECD principles for QSAR modeling to provide scientifically valid, high accuracy models with minimal complexity that support mechanistic interpretation, when possible. Existing OPERA models are also updated regularly. Recently, logKow, Fu and Clint models have been updated with the latest publicly available datasets to improve their predictivity and applicability domain coverage. OPERA also provides a tool for standardizing chemical structures, an estimate of prediction accuracy, an assessment of applicability domain, and experimental values when available. Technical and performance details are described in OECD-compliant QSAR model reporting format (QMRF) reports. OPERA predictions are available through the EPA CompTox Chemicals Dashboard and NTP's Integrated Chemical Environment. The OPERA application can also be downloaded from

NIEHS's GitHub repository as a command-line or graphical user interface for Windows and Linux operating systems. This project was funded with federal funds from NIEHS, NIH under Contract No. HHSN273201500010C. The views expressed above do not necessarily represent the official positions of any federal agency.

PO9

Application of systematic methods to characterize thyroid adverse outcome pathways (AOPs)

Daniele Wikoff[1], Stephen Edwards[2], Michelle Angrish[3], Peter Baumgartner[2], Ronnie Bever[4], Susan Borghoff[5], Grace Chappell[1], Rob Chew[2], Seneca Fitch[6], Ginnie Hench[2], Karen Hamernik[4], David Henderson[2], Andrea Kirk[7], Isabel Lea[8], Meisha Mandel[2], Lauren Payne[6], Andy Shapiro[3], Jon Urban[1], David Williams[2], Kristan Markey[4]
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Abstract

Systematic literature methods are increasingly used when developing AOPs; this includes evidence mapping to scope putative AOPs, followed by systematic review to improve the AOP definition and support evidence evaluation. We developed a stepwise process to systematically inventory and map biological information to a thyroid AOP network. The workflow involves two phases: 1) evidence mapping and 2) systematic review, each developed via a series of piloting and calibration exercises to ensure alignment with technical objectives and support development of data management and automation tools. The inventory phase was designed to broadly collect mechanistic information across multiple study types, species, and toxicological outcomes. Supervised machine learning tools suggested appropriate categories for manual reviewers to reduce the time required for the abstract review. Information can subsequently be mapped to potential key events (KE) in putative or established AOPs using a structured approach to inventory event components (e.g. object, process, action). This allows for stepwise interrogation of evidence by pathway, key event (such as

molecular initiating event), lifestage, species or other anchoring domain of interest. Systematic review can then be applied to further refine the AOPs, identify reference chemicals, or identify potential test methods. Collectively, these efforts will inform the development and evaluation of high-throughput assays for thyroid. The adaptation of systematic methods for AOP development demonstrates the utility of applying evidence-based approaches when defining mechanisms of toxicity.

The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the US Environmental Protection Agency.

PO10

Random Forest Models for Predicting Endocrine Activity: Advancing Methods for Screening and Identification of Endocrine Disrupting Chemicals

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Abstract

One of the current areas of concern in toxicology and chemical risk assessment are endocrine disrupting chemicals (EDCs), where computational toxicology can play an important role. The US EPA has run two programs, the Collaborative Estrogen Receptor Activity Project (CERAPP) and the Collaborative Modeling Project for Receptor Activity (CoMPARA), which aim to predict estrogen and androgen activity, respectively. The US EPA solicited research groups from the world for endocrine receptor activity QSAR models, which were combined to create consensus models for different toxicity endpoints. In an effort to build more robust models, in this work, random forest (RF) models were developed using large datasets from CERAPP and CoMPARA to predict estrogenicity and androgenicity, respectively. By utilizing simplistic descriptors and large training datasets, the RF models were created to screen chemicals rapidly and flag for potential endocrine disrupting activity. RFs were trained to conservatively predict the activity, meaning models are more likely to make False Positive predictions to minimize the number of False Negatives. Twelve unique RF models were created; binary and multi-class models to predict binding, agonism, and antagonism for estrogen and androgen receptors. The RF models presented were found to have

higher predictive capabilities than their CERAPP and CoMPARA consensus model counterparts, with some models reaching balanced accuracies of 93%. These models can be incorporated into evolving priority setting approaches to support screening and identification of chemicals for further testing and assessment by specifically flagging those that have the potential to be endocrine disrupting.

PO11

Machine Learning Comparison for Hepatoxicity Predictions Using Targeted Transcriptomic and Chemical Structure Data

Tia Tate, Grace Patlewicz, Imran Shah
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Abstract

Animal-based toxicity testing is both costly and time-consuming, making it difficult to keep up with the ever-increasing number of potentially harmful chemicals in the environment. Computational techniques that employ high-throughput experimental data can be used to analyze chemical toxicity more effectively. Several studies have shown that machine learning-based models can successfully predict substance toxicity using chemical structure descriptors, bioactivity descriptors from High-Throughput Screening (HTS) tests, and hybrid mixes of the two. Here, we used supervised machine learning to assess the feasibility of targeted high-throughput transcriptomic (HTTr) data in predicting in vivo repeat-dose hepatotoxicity. Furthermore, we assess the Generalized Read-Across (GenRA) approach, a similarity weighted method for generating automated read-across predictions, using F1 score, recall, and precision to compare this models performance to Artificial Neural Networks, Gradient Boosting, K-Nearest Neighbors, Logistic Regression, Naïve Bayes, Random Forest, and Support Vector classification algorithms. We identified target-specific classification accuracy for all algorithms, dependent on the imbalance of positive and negative compounds. Thus, several data balancing techniques were employed to objectively evaluate each algorithm's performance. Overall, we discovered that hybrid descriptors enhance hepatoxicity predictions (0.63 ± 0.16), particularly when performance bias owing to imbalanced data is addressed (0.73 ± 0.03). The Synthetic Minority Oversampling Technique (SMOTE) generated the most consistency in F1, recall, and precision improving overall performance by

62%. Furthermore, we discovered that GenRA performs as well as or significantly better than other classification algorithms for predicting in vivo toxicity results on unbalanced and balanced data. *This abstract does not reflect EPA policy.*

PO12

Systematic evidence mapping of research on environmental exposures and cardiovascular disease

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Abstract

Cardiovascular diseases (CVDs) represent a leading cause of mortality worldwide. Various factors influencing CVD have been relatively well-characterized e.g., lifestyle choices, genetic factors etc. Another potentially significant but underappreciated risk factor contributing to the development of CVD is environmental exposure to chemicals interfering with critical CV targets. The CV system is vulnerable to multiple environmental agents including pesticides, flame retardants, plasticizers, and air pollutants. There is mounting evidence that long-term environmental chemical exposure plays a significant role in progression of CVD. To better understand the landscape of environmental chemical influence on CVD, we developed a scoping review to systematically identify and categorize research reporting potential associations between environmental exposures and adverse cardiac outcomes. A comprehensive search was conducted in Pubmed that retrieved over 200,000 references. Given the particularly large number of references, iterative artificial intelligence algorithms were leveraged to prioritize and support manual title and abstract screening in Distiller, and machine learning approaches were used to facilitate categorization of references that reported data on cardiovascular outcomes after exposure to an environmental agent. Relevant references were characterized by evidence stream (human, animal, or in vitro exposure), study design, exposure, and major CV outcomes. An interactive evidence map was prepared to enable researchers to explore data rich and data poor areas in the literature by cardiovascular outcomes, environmental exposures, and other key factors. This map will inform evidence-based decisions

on the identification, selection, and prioritization of assay platforms and environmental chemicals that will be used by the DNTP to evaluate cardiovascular toxicity.

PO13

High-throughput screening to predict hERG inhibition

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Abstract

The human ether-a-go-go related gene (hERG) potassium channel plays a pivotal role in cardiac rhythm regulation. Inhibition of hERG channels can lead to a prolongation of the QT interval. Environmental toxicants have the potential to contribute to the pathophysiology of complex diseases, but the underlying mechanisms remain obscure. To date, more than 100,000 chemicals have been introduced into commerce with limited toxicological testing. An evaluation of the effect of environmental chemicals on hERG channel function can help inform the potential public health risks of these compounds. To assess the effect of environmental chemicals on hERG channels, the US federal Tox21 program has screened a collection of 9667 chemicals using a cell-based thallium-influx assay in U2OS cells stably expressing hERG in a quantitative high-throughput screening (qHTS) format. The chemical results in the hERG qHTS assay were characterized using a set of molecular descriptors, physicochemical properties, Self-Organizing Maps (SOM) and hierarchical clustering. Statistical machine learning approaches were applied to build quantitative structure–activity relationships (QSAR) models to predict the probability of a chemical to inhibit hERG in this thallium flux assay, applying both classification and regression techniques. Models were compared with existing QSAR hERG models and dataset. The evaluation of performance criteria of generated models revealed that Random Forest model outperforms other models. This tiered clustering and predictive modeling approach facilitates detection of environmental chemicals that merit more extensive evaluation for

cardiotoxicity and provides useful structural information that could be applied to predicting the potential for new chemical entities to inhibit hERG.

PO14

Optimization of random-forest machine learning methods using structural fingerprints to predict binding to the estrogen receptor alpha

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Abstract

We mined public *in vitro* high throughput screening data in ToxCast and ChEMBL to develop computational prediction models that predict whether a compound will [positive] or will not [negative]) bind to the estrogen receptor alpha (ERα). Using the open-source KNIME platform, we investigated the impact of several parameters on model performance: database size; use of structural keys (MACCS and/or PubChem) or Morgan extended connectivity fingerprints with variation of atomic radius size; use of conjoint keys/fingerprints; and number of decision trees in a forest. The database comprised competitive binding assay data for ERα and contained 1608 positive and 1796 negative compounds. Each tree node used a random bootstrapped sample of 60-65% of the database plus a resampled replacement sample. Input features were a random subset of structural bits = SQRT total bits, with the most informative bit chosen per node. Predictions were based on consensus among the trees. Model statistics improved remarkably with database size. Sensitivity, specificity and balanced accuracy were > 91% using a single decision tree and improved with increasing number of trees (10, 100, 1000), with specificity approaching 100%. All structural keys and fingerprints performed well, as did conjoining them in several combinations. Morgan fingerprints preliminarily performed bests when the atomic radius was set to 2. To assess potential disruption of endocrine signaling, we advocate an integrated approach with initial *in silico* screening for potential binding to ERα. Future research shall extend these queries to understand the generality of the findings for ERα to other molecular targets.

PO15

Validation of the ToxProfiler reporter assay for toxicological profiling and determination of the underlying mode of action

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Abstract

Classical in vitro chemical safety assessment often relies on simple cytotoxicity endpoint measurements. However, the underlying mechanisms are usually poorly understood from these general assays. In order to gain further understanding of cell stress mechanisms we have developed the ToxProfiler assay, a unique new approach method (NAM). ToxProfiler can be applied to accurately quantify chemical-induced stress response pathways at a single cell level to reveal the toxicological Mode of Action (MOA) of novel medicines, (agro)chemicals, cosmetics and food ingredients.

ToxProfiler consists of a collection of 7 unique genetically engineered human liver HepG2 cell lines. Each cell line contains a green fluorescent protein reporter that reflects a specific cellular stress response pathway; oxidative stress, genetic stress, endoplasmic reticulum stress, ion stress, protein stress, autophagy and inflammation. To visualize activation of the different cellular stress response reporters, we developed an automated live cell confocal imaging and data analysis pipeline to derive a unique quantitative toxicity fingerprint of chemicals. A point of departure (POD; lowest concentration at which a significant response was observed) is determined for each endpoint in order to directly compare the potency from one chemical to another. Here we present the validation of the ToxProfiler technology including assessment of specificity.

To summarise, we developed and validated a new mechanism-based in vitro reporter assay, ToxProfiler, that can provide mechanistic toxicity information for early chemical safety testing, read-across, adverse outcome pathways (AOP) and weight-of-evidence (WoE) approaches.

PO16

Multi-scale comparative analysis of the mechanisms of organophosphorus pesticide developmental neurotoxicity

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Abstract

Organophosphorus pesticides (OPs) are a chemically diverse class of commonly used insecticides. Inhibition of acetylcholinesterase (AChE) as the shared mechanism of acute OP neurotoxicity is well studied. However, it has been suggested that chronic low-dose exposure to OPs causes developmental neurotoxicity (DNT), via interactions with alternative targets. Because most studies have focused on chlorpyrifos, it remains unclear whether different OPs act through different mechanisms. We hypothesized that differences of OP DNT are due to differential effects on alternative targets. To test this, a comparative high-throughput screen of 7 OPs (acephate, chlorpyrifos, dichlorvos, diazinon, malathion, parathion and profenofos) across 10 concentrations in quarter-log steps was performed. Asexual freshwater planarians were used because this invertebrate system uniquely allows for testing of adult and developing specimen in parallel on an automated system. Twenty-two "mechanistic control compounds" known to target pathways suggested in the literature to be affected by OPs, and assay negative and positive controls were also tested. Neurotoxicity was quantified across 29 (morphological and behavioral) readouts using automated image analysis. Phenotypic barcodes were created to quantify the holistic toxicological profile for each chemical concentration using the standardized, compiled (n=24 planarians) quantitative scores for each of the 29 endpoints. Multidimensional scaling revealed that the OPs separated into mechanistic clusters. The phenotypic profiles of adult vs regenerating planarians exposed to the OPs clustered differently, suggesting some developmental-specific mechanisms. This study provides new mechanistic insight into how OPs differentially damage the developing brain. Supported by NIH grant R15 ES031354.

PO17

The Predictive Analytics Toolkit and "Big" Data

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Abstract

The Predictive Analytics Toolkit (PAT) was developed to facilitate use of new approach methodologies (NAMs) to predict hazard and risk. PAT is a user-friendly web application that provides access to many R packages to enable development and testing of prediction models. Here, we discuss PAT's features and describe using PAT with "big" data. We drew from the work of Ring et al. 2021 (doi:10.1016/j.comtox.2021.100166), who used random forest models to predict in vivo transcriptomic responses in rat liver for a set of 221 chemicals using 12 different models that varied in the inclusion and type of chemical disposition and toxicokinetic metrics of Tox21 AC50 values. Gene ontologies helped identify 735 biological pathways based on differential in vivo expression of specific gene sets. Ring et al. used these 12 models to predict in vivo activity using 5000 random forest iterations for each chemical/pathway combination. The area under the receiver-operator characteristic curve (AUC-ROC) was the measure of model performance. The model with the highest AUC-ROC for a given pathway was considered the "winner." Using a subset of 10 pathways from the Ring et al. data, we used PAT to predict the AUC-ROC and to compare the best (Model 10) and worst (Model 2) models with only 100 random forest iterations. Ring et al. reported Model 10 "won" in 52.2% of the comparisons. Using the results from PAT, Model 10 "won" in 60% of the comparisons. Hence, PAT may provide a useful alternative to programming in R, even for "big" data.

PO18

Animal-free preclinical cardiac risk evaluation on chronic level using the high throughput FLEXcyte technology

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Abstract

In preclinical drug development, cardiac contraction analysis of drug candidates is one of the crucial steps to ensure a successful transition to clinical stages. The use of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) continues to increase as an animal-free approach for assessing safety and toxicological side effects of newly developed compounds. However, limited timescales (min to h) after compound application remains the primary application so far, due to the inability of common cell-based assays to analyze cellular behavior reliably over prolonged periods of time.

Here we describe the bio-compliant 96-well FLEXcyte technology and its applicability for chronic cardio-toxicological studies. Commercial hiPSC-CMs were cultured on freely-swinging and hyper-elastic silicone membranes. Rhythmic contraction of hiPSC-CMs resulted in dynamic deflection changes quantified by capacitive distance sensing. The resulting beat patterns were analyzed for essential inotropic parameters including amplitude, frequency, slopes of contraction and relaxation, area under curve and arrhythmic events.

We selected 15 kinase inhibitors and 3 anthracyclines with well-known cardiotoxic profiles to evaluate the reproducibility of clinical data. For the assessment of chronic compound effects, inotropic properties of the cells were recorded daily for five days. Compounds considered as clinically cardio-safe showed negative inotropic effects only at micromolar doses, while compounds with demonstrated cardiotoxic profiles showed both time and dose dependent inotropic effects as well as arrhythmic events at nanomolar concentrations.

Our results indicate that the FLEXcyte technology enables the assessment of animal-free and physiologically relevant inotropic effects on chronic level beyond the current perspective of preclinical cardiac risk assessment.

PO19

Accelerating adoption of NAMs with FAIR principles

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Abstract

Riding on the coattails of "omics" and "big data" are concepts like "ontologies" and "FAIR data". But what do these mean and how are they relevant to the day-to-day work of scientists and regulators working on chemical health and safety? This presentation introduces key concepts in FAIR (findable, accessible, interoperable, and reusable) data principles for data creators and consumers along with answers to the questions "why should I care?" and "how can I incorporate them into my research?" We will show case studies on how "little" data sets common to toxicology become "big" data and how various "omics" studies become pathways to insight using FAIR annotation. The impact of improving data FAIRness on the assessment and use of new approach methodologies (NAMs) will be discussed. Case studies presented will draw upon data resources from the National Toxicology Program such as the Chemical Effects in Biological Systems (CEBS) and the Integrated Chemical Environment (ICE) which are undergoing efforts to adopt and promote FAIR principles. This project was funded with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.

PO20

In vitro to in vivo extrapolation for developmental toxicity potency of selected Tox21 chemicals

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Abstract

To support the implementation of new approach methodologies for regulatory decision-making on developmental toxicity, 186 chemicals were tested in a human induced pluripotent stem cell-based assay, devTOX *quick*Predict

(devTOX^{qP}). In this study, we evaluated the performance of the devTOX^{qP} assay for predicting the lowest effect level (LEL) in rat developmental toxicity studies. We performed *in vitro* to *in vivo* extrapolation (IVIVE) using the developmental toxicity potential (dTP) concentration from the devTOX^{qP} assay to estimate equivalent administered doses (EADs) that would result in the maximum plasma concentrations equivalent to dTP concentrations. The resulting EADs were compared to *in vivo* LELs. Additionally, we evaluated the impact of *in vitro* kinetics, pharmacokinetic parameters, and different physiologically based pharmacokinetic (PBPK) models on EAD estimates. Our preliminary results showed that the EAD estimates using an open-source, generalized PBPK model are lower than the rat developmental toxicity LELs for approximately 70% of chemicals, suggesting that human cells are more sensitive and devTOX^{qP} assay may provide a more conservative hazard estimate for use in risk assessment. The fold differences between EAD estimates and rat LELs vary among chemicals. For over half of the chemicals tested, EAD estimates are within an order of magnitude of the lowest LELs. Adjusting for *in vitro* kinetics can improve prediction for rat LELs for some, but not all chemicals, indicating a need for further characterization of conditions when this adjustment should be applied. This project was funded with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.

PO21

In Silico Assessment of Dermal Toxicity Considering Exposure, Hazard, and Potency

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Abstract

Providing alternatives to animal testing is required in the European cosmetics market as well as in several states in the United States. Dermal toxicity is an important endpoint where a series of events after chemical contact on the skin can be assessed by in vitro and in silico approaches. Our “In Silico Skin Suite” considers three events: 1) exposure through skin permeability; 2) chemical

reactivity and toxicity manifested by skin irritation; 3) skin sensitization leading to contact dermatitis. In silico approaches, based on chemical structure and physicochemical properties, are combined with experimental results from in vitro assays. For skin sensitization, computational and experimental strategies are designed to reflect key events in the adverse outcome pathway (AOP), in which the molecular initiating event (MIE) is the covalent modification of proteins. ToxPrint chemotypes, structural fragments encoded with physicochemical properties and electronic system information, were used to categorize chemicals into MIE classes. Both structural rules and quantitative structure activity relationship (QSAR) models have been constructed to model these events. For assessment of irritation and sensitization, a weight of evidence approach was used to combine multiple sources of information and assess uncertainties. To give an assessment of hazard and potency, Dempster-Shafer Decision Theory and Bayesian Network approaches were considered. This talk presents an overview of the approach whilst a detailed case study demonstrated in ChemTunes•ToxGPS® is presented in an accompanying poster.

PO22

Improving Quality of In Vitro Methods: A GIVIMP Certification Program

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Abstract

In 2018 the Organization for Economic Cooperation and Development (OECD) issued a Guidance Document on Good In Vitro Method Practices (GIVIMP). Intended to reduce the uncertainties in cell and tissue based methods used in the prediction of human safety, GIVIMP provides the field with a set of standards to improve both the quality and accuracy of newly developed, and routinely executed, *in vitro* methods. Currently organizations are struggling to define the best approach for practical implementation of the guidance within their programs. As GIVIMP is broad in scope and covers a wide range of scientific and quality topics, there is the potential for varying interpretations of the guidance and thus significant differences in implementation. A business to business certification program is one solution to harmonize GIVIMP interpretation, standardize “claims” of compliance with the document, and provide a uniform

roadmap for incorporation of GIVIMP principles within routine laboratory operations and method development activities. A certification program would be beneficial to the full audience for which GIVIMP was intended including academic laboratories developing new methods, established laboratories participating in validations and/or performing routine *in vitro* studies, and industry laboratories intending to submit *in vitro* data to regulatory agencies. A pilot certification between the Institute for In Vitro Sciences (IIVS) and BASF SE Experimental Toxicology and Ecology laboratories (Ludwigshafen, DE) has been launched to provide proof-of-concept for the program. This poster discusses the need for the GIVIMP certification program and provides details on its structure and administration.

PO23

Incorporating *in silico* methods in weight of evidence approaches

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Abstract

In silico methods can play an important role in deriving a weight of evidence assessment. In such assessments, an important consideration is how much weight can be placed on an *in silico* prediction; particularly, when integrated with additional data. Addressing this question requires evaluation of the reliability and relevance of *in silico* predictions in a manner that is analogous to experimental results. *In silico* protocols which describe the use and evaluation of *in silico* methods to support various toxicological endpoints are being developed and implemented such that the overall confidence in the assessment can be communicated. In this presentation, we discuss the framework to support the development of these *in silico* protocols, and demonstrate how to assign reliability scores, assess relevance, and derive the level of confidence in Quantitative Structure Activity Relationship (QSAR) assessments to determine skin sensitization hazard. Examination of these parameters is important to understand how much emphasis could be placed on an *in silico* model's result in a weight of evidence scenario.

PO24

Opportunities for reduction and refinement of animal use in 26-week carcinogenicity studies in TgrasH2 mice

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Abstract

For 158 US FDA reviews of New Drug Applications approved in 2015 through 2019, we compared information submitted by sponsors to ICH recommendations. In 64 reviews, FDA reported submission of 2-year studies in mice or rats or 26-week studies in rasH2 mice. The most common reasons sponsors gave for waiving studies were indication, duration of use, and no cause for concern. Sponsors reported carcinogenicity in mice or rats in 17 NDAs; the most common reason FDA gave for approval was lack of mechanistic relevance. The most common testing strategy used was 2-year studies in both mice and rats followed by a 2-year study in rats plus a 26-week study in rasH2 mice. 23,857 mice were used in 32 2-year studies (745 mice per), 30750 rats were used in 51 studies (603 rats per), and 9,142 rasH2 mice were used in 23 26-week studies (208 mice per). The greatest reduction would be achieved with microsampling to evaluate toxicokinetics, reducing or eliminating animal use in TK satellites; this would be greatest for mice, who are killed to collect conventional sample volumes. A significant reduction would be achieved by using single, rather than dual, negative controls, as recommended by ICH. While FDA reported the use of dual controls, it found both single and dual control studies to be adequate. A smaller reduction would be achieved with genotyping, reducing or eliminating the use of mice in positive controls for 26-week studies. Poster and abstract were revised to address opportunities for reduction in all studies.

PO25

New Approach Methods' Model Analysis and Adverse Outcome Pathway Development Validating Retinoid Signaling Associated with Skeletal Dysmorphogenesis

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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. 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. Here, a data-driven model was constructed to identify chemicals associated with both ATRA pathway bioactivity and prenatal skeletal defects. We identified altered skeletal phenotypes in prenatal developmental toxicity studies in ToxRefDB and/or ToxCast high-throughput screening (HTS) and identified 375 chemicals associated with the alterations. Defects were organized into four skeletal phenotype groupings: cranial, post-cranial axial, appendicular, and other non-specified skeletal defects. To build a multivariate statistical model, HTS results from >8,070 chemicals in ToxCast/Tox21 across 13 *in vitro* assays, representing key nodes in the retinoid signaling system were evaluated and compared to candidate reference chemicals for *in vitro* testing. There were 52 chemicals were identified for constructing data-driven models to link this *in vitro* data with adverse skeletal outcomes for computational modeling. These preliminary findings will guide the development of dynamic modeling and AOPs for mechanistic validation to strengthen evidence for causality. Furthermore, NAMs identified 27 without previous evidence of retinoic acid pathway disturbance and skeletal defects. These findings shed light on potential avenues for new mechanistic discoveries related to retinoic acid pathway disruption and associated skeletal dysmorphogenesis. *This abstract does not represent the official views of EPA or any government agency.*

PO26

Retrospective Clinical Evaluation for the Development of Reference Chemical Lists

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Abstract

As new approach methodologies (NAMs) continue to be developed and adopted, an ongoing challenge for *in vitro* cell and tissue culture assays is defining appropriate approaches to validation. A major advantage of human cell and tissue culture models is the ability to improve the accuracy of translation of results from the research bench to real-world scenarios. However, limitations can emerge during validation if gathering prospective clinical data is not possible, especially for hazardous chemicals. Therefore, practical approaches to develop and maintain the most clinically relevant data for comparison are needed. To this end, information from case studies can be evaluated retrospectively to identify and characterize the hazards associated with chemical use in real-world conditions. Herein we describe such an approach for the identification of respiratory sensitizers, in which we utilize the EPA-developed Abstract Sifter literature review tool and standardized search terms to maximize the retrieval of publications relevant to respiratory chemical allergy or asthma in humans. This approach successfully identified over twenty compounds as known respiratory sensitizers based on well-defined clinical diagnostic criteria. This output will be used along with other available data to establish a reference list of respiratory sensitizers, irritants, and non-sensitizers, to update existing risk assessment approaches and evaluate the accuracy of new approaches for this key endpoint. Overall, this approach provides an exemplary method to evaluate and apply human clinical data as part of the weight-of-evidence towards establishing reference chemical lists. *This abstract does not necessarily represent U.S. EPA policy.*

PO27

Release of Population Life-course Exposure to Health Effects Model (PLETHEM): An Online Tool for PBPK Modeling version 3.0

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Abstract

An outstanding challenge in the acceptance of alternatives to animal testing is the systematic incorporation of computational models into risk-based decision-making pipelines. This can be achieved by linking exposure estimation methods,

physiologically based pharmacokinetic (PBPK) modeling, and computational systems biology pathway modeling tools into a standardized framework. To that end, we have developed the Population Life-course Exposure to Health Effects Model (PLETHEM) suite, a modular open source modeling platform that provides users the ability to create, run, share, and audit PBPK models. The platform consists of a database of chemicals, QSAR models, life-stage specific physiological and metabolic parameters needed to parameterize PBPK models, an R-based engine to perform model simulations, and an interactive user interface to define and select parameter sets for the models. PLETHEM implements easy to use interfaces for a generic PBPK model and a high-throughput IVIVE model. The most recent iteration of PLETHEM updates the ecotoxicology module to increase stability and to include additional modeling output. The rapidPBPK module has been overhauled to increase stability and throughput and to include a batch mode and sensitivity analysis capabilities. PLETHEM is available online at www.scitovation.com/plethem.

PO28

Proposing Known Respiratory Sensitizers as "Gold Standard" for Researching Novel Approaches on Respiratory Sensitization – A focus on low molecular weight chemicals

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Abstract

Background: The induction of immunological responses in the respiratory tract due to previous exposure to a compound is known as respiratory sensitization, and the compound is known as a respiratory sensitizer. However, the variability of the symptoms presented in humans from occupational inhalation exposures to low molecular weight chemicals, the limited available evidence, and the absence of standardized and validated test models hinders the identification of true respiratory sensitizers. As such, it is important to utilize an appropriate positive control for respiratory sensitizers when developing scientific projects on respiratory sensitization.

Objective: This project aims to categorize the known low molecular weight respiratory sensitizers based on compelling, reasonable, inadequate, and

questionable evidence in humans and identify the chemical respiratory sensitizers as the 'Gold Standard' or reference list for scientific research purposes.

Methods: An inventory of 95 known respiratory sensitizers was generated from 5 resources, of which 50 low molecular weight organic chemicals were subjected to literature review. These chemicals were categorized based on the set criteria as having compelling, reasonable, inadequate, and questionable evidence in humans from occupational inhalation exposures. Of these 50, three chemicals' literature searches did not reveal any relevant data and therefore could not be assigned to either of the four categories.

Results: Less than 10 chemicals were confirmed with compelling evidence in humans as a reference list for low molecular weight respiratory sensitizers, with specific anhydrides and diisocyantes dominating the category.

PO29

Application of in vitro 3D Reconstructed Human Epidermis Models EpiKutis® and EpiDerm™ to Predict Skin Irritation Potential of Surfactant Based Formulations with Different Physicochemical Properties

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Abstract

Background: Our previous study indicated both EpiDerm™ and the China-sourced *in vitro* Reconstructed Human Epidermis Model EpiKutis® can detect the effect of surfactant concentrations on skin irritation in a dose-response manner. **Objective:** We aim to use EpiKutis® and EpiDerm™ to predict skin irritation of six structurally different surfactants in final formulation. **Methods:** Six

rinse off formulations with the same base with a one surfactant difference (four anionic, one zwitterionic and one nonionic) were tested utilizing EpiKutis® (Guangdong BioCell Biotechnology, Co. Ltd., China) in comparison to the well-established EpiDerm™, following a time to toxicity screening approach. The base formulation containing a mixture of two surfactants was also tested. The same base without surfactant was used as negative control. Previous findings indicated optimal dilutions as 10% for EpiDerm™ and 5% for EpiKutis®. **Results:** Results are comparable for both tissue models, supporting the reliability of skin irritancy *in vitro* predictability. The results also show the surfactant physicochemical properties influence the irritation profile. The ET50 indicates skin irritation increases in the sequence of nonionic, zwitterionic and anionic. **Conclusion:** This study, alongside a previous study, demonstrate consistency in exposure methods and dilutions in both models to obtain reliable results to determine skin irritation. The *in vitro* skin irritation results also show that the mild nature of surfactant-based formulations correlate with the concentration and physicochemical properties of the surfactant. The results demonstrate both models are predictive for skin irritation and present opportunity for use with other cosmetic product forms.

PO30

Analyzing multi-dimensional developmental neurotoxicity new approach methodologies: computational approaches to identify phenotypes

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Abstract

Current developmental neurotoxicity (DNT) hazard assessment relies on *in vivo* testing that is resource intensive and lacks information on key cellular processes affected by chemical exposures. To address these limitations, DNT New Approach Methodologies (NAMs) are being evaluated for their utility to inform DNT hazard, including: 1) functional microelectrode array network formation assay; and 2) high-content imaging to evaluate proliferation, apoptosis, neurite outgrowth, and synaptogenesis. This work applies computational approaches to

address three related hypotheses for a 92 chemical dataset: (1) a broad screening battery will provide a sensitive marker of DNT bioactivity; (2) evaluating selective bioactivity (below the cytotoxicity threshold) may provide a more specific indicator of the functional processes underlying DNT; (3) some subset of endpoints may optimally classify DNT reference compounds. Hierarchical clustering of the potency and 'selectivity' values revealed that potency was sufficient to capture any effect on DNT-relevant processes but did little to distinguish patterns of neural network function, while selectivity revealed compounds with distinct, differential DNT-relevant activity. The potency analysis classified DNT reference compounds with a higher sensitivity than the selectivity analysis (83% versus 74%) indicating that cytotoxicity is informative for classifying DNT positives. The false negatives appeared to be associated with several limitations, such as maximal concentration tested or gaps in the biology of the battery. This study emphasizes the importance of an integrated analysis that combines computational approaches, a broad chemical set, and a diverse suite of assays to demonstrate the fit-for-purpose utility of DNT NAMs. *This abstract does not reflect EPA policy.*

PO31

Policy Initiatives for Integrating New Approach Methodologies for Testing Pharmaceuticals in the United States

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Abstract

New approach methodologies (NAM) that utilize human cells and tissues are expected to better protect public health than traditional nonhuman animal-based approaches by providing toxicity information that more accurately predicts human response. Additionally, the United States Congress has expressed the desire for improved integration of NAMs in pharmaceutical development due to the scientific benefits of NAMs and ethical concerns of animal testing. To support the integration of NAMs for testing pharmaceuticals, policies outlining drug sponsors' requirements and expectations must clearly allow for NAM use. This

level of regulatory certainty is critical because traditional animal studies are otherwise ingrained in regulatory policy and industry practice.

Multiple policy initiatives in the United States are moving toward NAM integration, including the FDA's launch of the Innovative Science and Technology Approaches for New Drugs (ISTAND) pilot program which provides a pathway for regulatory acceptance of NAMs. A method qualified for a context of use under ISTAND can be included in regulatory submissions for the context of use without the need for FDA to reconsider and reconfirm its suitability, providing regulatory certainty to drug sponsors. Opportunities remain for updating the regulatory framework. For example, many regulations require nonclinical animal data. To account for evolving NAM development, requirements should be neutralized by changing references from "in vivo" and "animal" to "nonclinical," which encompasses in vivo, in vitro, and in silico approaches.

PO32

Seq2Fun: Software for Ultrafast and Species-Agnostic Functional Profiling of RNA-Seq Data

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Abstract

Conducting cross-species extrapolation of 'omics data is critical for using toxicogenomics data to inform environmental risk assessment. Prior to real-world application, we must have a deep understanding of how whole-transcriptome profiles compare across many species to identify both opportunities and limitations of cross-species extrapolation. This is time consuming and complex, with challenges including replicate genes from genome duplications, one-to-many ortholog mapping, and de novo transcriptomes that have hundreds of thousands of poorly annotated transcripts. Here, we introduce Seq2Fun (www.seq2fun.ca), a reference-free and species-agnostic tool for RNA-seq quantification. In a case study, we demonstrate how it can be used to simplify cross-species comparisons of RNA-seq profiles. Seq2Fun works

by translating reads into amino acid sequences and then directly mapping them to the KEGG database of protein sequences, resulting in a counts file that is expressed in terms of KEGG ortholog (KO) IDs. Because each read is processed individually, Seq2Fun is >120 times faster than de novo transcriptome assembly and can be run on a standard desktop computer with low RAM requirements. Since Seq2Fun maps reads to the same set of KOs regardless of species, the results are well-suited for cross-species comparisons. In the case study, we compare RNA-seq profiles from multiple life stages of six species that were exposed to the same set of eight environmental contaminants (n = 674 profiles; collected as part of the EcoToxChip project). In the analysis, we 1) identify species and life stage-specific KOs; 2) compute relative conservation of KO expression at the pathway level; and 3) compare transcriptomic responses to the same chemical across different species. In doing so, we show how Seq2Fun reduces barriers for cross-species comparison of whole-transcriptome profiles.

PO33

General Signature of Genotoxicity: Evaluation through meta-analysis of gene expression studies

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Abstract

Identification of carcinogenic hazard and discrimination between genotoxic and non-genotoxic carcinogens are important components of chemical risk assessment; however, it remains challenging due to the recognized limitations of traditional genotoxicity assays, including conflicting results. We have previously derived a General Signature of Genotoxicity (GSG) based on the systematic review of previously published in vivo transcriptomic studies involving several different tissues and animal species. The GSG consists of genes that tend to display up-regulation following genotoxic challenge that have differing degrees of conservation of response across tissues. In this study, we examined changes in expression of the GSG genes after various genotoxic and non-genotoxic exposures, using the in vitro and in vivo data accessed through the BaseSpace

Correlation Engine (Illumina). We demonstrate that individual GSG genes and their combinations display considerable differences in their predictive performance and we define data-driven applicability of the GSG for its use to predict genotoxicity from transcriptomic data. Our results support potential of the GSG to inform the chemical risk assessment. Disclaimer: The views expressed are those of authors and do not necessarily represent the views or policies of the U.S. Government and they may not be used for advertising or product endorsement purposes.

PO34

Identify endocrine disrupting chemicals using a suite of complementary *in silico* models

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Abstract

There is a need for new approach methodologies (NAMs) to identify endocrine disrupting chemicals involved in key events of endocrine pathways including binding to receptor proteins. In 2018 a guidance describing how to perform hazard identification for endocrine-disrupting properties by following the scientific criteria which are outlined in EU 2017/2100 and EU 2018/605 for biocidal products and plant protection products, respectively, was published by ECHA and EFSA. In this guidance computational approaches are proposed as line of evidence for endocrine activity assessment. An *in silico* screening workflow which follows this guidance is described in this study. It employs 108 freely available and commercial models covering 27 receptors: 62 (Q)SARs, 19 rule-based profilers, 18 models of receptor interactions and 6 ToxCast pathway models. All the models are applied and the results are combined with an algorithm that considers existing experimental data, the reliability of the model and the uncertainty of each prediction. The predictions are then combined by receptor and an overall conclusion for each receptor and is given. When models with the same level of uncertainty disagree an expert assessment of the nearest neighbours is carried out to get to a final conclusion. Positive predictions are used to give indication on the mechanism of action for the endocrine disruption. The workflow was implemented in KNIME. Two examples are

demonstrated: butylparaben, correctly predicted as active towards the estrogen receptor, and triclosan, correctly predicted to be active towards the androgen receptor and towards the constitutive androstane receptor.

PO35

An Adverse Outcome Pathway Describing How Inhalation of Airborne Chemicals Leads to Decreased Lung Function

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Abstract

Lung surfactant is a thin liquid film covering the inner surface of the alveoli, and primarily functions to regulate the surface tension during breathing by forming complex structures of phospholipids and surfactant-associated proteins. The lung surfactant is the first biological entity met by inhaled substances reaching the alveoli. The interaction with lung surfactant can either cause little to no consequence or inhibit lung surfactant function. The presented adverse outcome pathway (AOP) focuses on the inhibition of lung surfactant function by the inhaled substance as the molecular initiating event (MIE). Inhibition can lead to adverse outcome (AO), decreased lung function, via a series of key events (KE). The inhibition of lung surfactant function leads to high surface tension at the maximum compression of the film, i.e., at the end of expiration, and alveolar collapse. The re-opening of the collapsed alveoli on inspiration causes shear stress on cells covering the alveoli and damages the alveolar-capillary membrane integrity allowing blood components to flow into the alveolar airspace. Alveolar collapse is responsible for a decrease in the surface area for oxygenation of blood and reduced tidal volume. The KEs result in decreased lung function characterized by clinical signs of toxicity such as shortness of breath, coughing, and troubled breathing.

In vitro measurement of lung surfactant function, results from testing different chemical entities (e.g. impregnation products, inhaled pharmaceuticals) are presented together with the weight of evidence analysis for the AOP. This AOP is #302 in the AOPwiki.

PO36

ToxEraser: A New Tool for Substitution, Towards Safer Cosmetic Ingredients

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Abstract

At the core of the sustainable cosmetology and the green chemistry there is the concept that throughout the development and production of chemicals, risk should be minimized. Replacing harmful substances with less harmful ones is widely acknowledged as a very effective strategy to reduce, minimize, or even eliminate risks.

Within the LIFE VERMEER project (https://www.life-vermeer.eu/), a new, free-to-use software has been developed with the objective to provide an innovative and user-friendly way to substitute risky chemicals by a systematic search of potential alternatives. This tool, called ToxEraser, is able to identify risky cosmetic ingredients suggesting safer compounds as remedy. It has been designed to highlight possible safer alternatives among ingredients manufactured for the same practical scope. Key information about safety of each item concerns: (a) the risk assessment addressed by seven Regulatory and other specialized European-US authorities; (b) the final safety class emerging from the systematic evaluation and integration of each authority assessment. Read-across analysis enforces the substitution process. The list of alternatives can be extended or reduced flexibly, since the commercial use of interest is specified by attributes indicating progressively refined and hierarchically related categories, detailing the functional use of the ingredient.

The tool has been designed to be joined with SpheraCosmolife, the other tool developed within the VERMEER project and recently published by the authors. ToxEraser will offer a systematic and flexible perspective to explore safer cosmetics substitutes, by acknowledging the sources of evidence produced by SpheraCosmolife, offering a forward-looking tool for the cosmetic sector.

PO37

Development of PLHC-1 multicellular spheroids as in-vitro models to assess the mixture toxicity of plastic additives

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Abstract

Plastic pollution creates enormous challenges, including evaluating its toxicity and possible adverse effects on aquatic organisms, which are constantly exposed to complex mixtures of plastics and plastic additives. This work investigates the molecular and cellular responses generated by a mixture of 10 common plastic additives -several bisphenol A derivatives, phthalates, alkylphenols, triclosan and tritolylphosphate- tested at a equimolar concentration in *Poeciliopsis lucida* hepatocellular carcinoma (PLHC-1) cell line cultured in monolayer. Significant cytotoxicity and generation of reactive oxygen species (ROS) was observed for the mixture at much lower concentration - 1.5 µM and 0.5 µM - in comparison to single compound exposure. In addition, we employ PLHC-1 multicellular spheroids as a testing platform that better simulates the microstructure and environment of cells in vivo. Spheroids from days 2-8 and size of 150 - 250 µm maintained healthy and stable. The lipid signature of PLHC-1 cells analyzed by flow injection coupled to high resolution mass spectrometry (FIA-ESI(+/-)-Orbitrap-Exactive) evidenced that when moving from monolayer to microtissues, acylglycerols increased significantly. These changes were associated to the development of a liver-like phenotype and possible the accumulation of lipid droplets in the spheroids. Overall, this study highlights the need of studying mixture toxicity, and proposes the use of fish liver spheroids as a model in aquatic toxicology studies.

PO38

New spherical harmonic based descriptors to efficiently fuel QSAR methodology: Endocrine disruptor case study

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Abstract

Two-dimension quantitative structure-activity relationship (2D QSAR) has been a standard methodology for the last decade whereas multiple three-dimension (3D) descriptors have been tested with mitigated successes. In the present study, we propose a new set of highly informative and compact 3D descriptors from spherical harmonic (SH) based representations covering both the geometrical shape and the pharmacophoric features of a molecule. The process consists in placing a molecule on three different axes – each one is captured by its own set of spherical harmonics. SH related expansions are used to create compact and rotation independent descriptors - e.g. 32 floating coefficients - to describe a conformer of the molecule. These descriptors were then applied to a QSAR model of toxicity which was built from the reference dataset of the CERAPP project - a collaborative project that developed a consensus model of toxicity for the endocrine disruption [Mansouri *et al.* 2016]. The QSAR model was trained with SH based descriptors through a random forest algorithm and binding activity to the estrogen receptor was considered in this study. The resulting model yielded a balance accuracy of 0.87 on the evaluation dataset. Furthermore, by combining SH and 2D descriptors from the RDKit suite [http://www.rdkit.org], the subsequent QSAR model gave rise to a balance accuracy of 0.91 on the evaluation dataset, positioning its performance at the high level of the consensus model obtained by the CERAPP project.

PO39

Developmental Neurotoxicity Testing: Generation of fluorescent brain organoids for oligodendrogenesis and myelination quantification.

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Abstract

Developmental Neurotoxicity Testing (DNT) is one of the most complex endpoints to address. Current in vivo approaches are prohibitively expensive, time consuming and have low predictivity for humans. Therefore, a battery of DNT tests has been developed to screen the potential DNT toxicants in vitro. Oligodendrogenesis and myelination are key events of neural development, which arecovered in this battery. However, it is on of the most complex processes to model in vitro. Therefore, we are developing an assay to

quantify oligodendrogenesis and myelination in 3D brain organoids (BrainSpheres). 3D brain cultures have several advantages over traditional monolayer cultures, especially for such complex processes as myelination.

Here we described an improved protocol to transfect human immortalized pluripotent stem cells (hiPSC). We take advantage of CRISPR technology to insert a fluorescent tag in the PLP1 gene, an oligodendrocytes marker. Transient puromycin selection was performed to enrich the population of template donor-plasmid carriers. Single cell colony formation assay was performed as well as screening and validation of the clones. We considerably improve overall knock-in efficiency as well as homozygous knock-in efficiency compared to previous reports. The selected clones were differentiated to neural progenitors and brain organoids for validation of the tag expression and specificity by flow cytometry and confocal imaging. Overall, these results provide a new method to follow oligodendrocytes' differentiation and maturation during in vitro neurodevelopment together with a more effective method to generate reporter cell lines, which can be used in numerous applications including (developmental) neurotoxicity screenings.

PO40

Toxic effects from coal mines: A review

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Abstract

Background: epidemiological studies showed the evidence of the association of health risks in general population working in coal mines. The international classification of diseases (ICD) can be used to compare the findings associated with socioeconomic and geographical contexts.

Objectives: using ICD codes in studying morbididty and mortality in population from coal mines.

Methods: A systematic database search was carried out from 1990-2021. The health outcomes were mapped according to ICD.

Results: Among the studies selected the exposed population had increased risk of mortality and morbidity. Neoplasms, diseases of the circulatory, respiratory and GI disorders, chromosomal abnormalities were common among these studies. The risks associated with genitourinary system and prostate cancer was common and predominant.

Conclusion: there is consistent evidence of association of coal mining with wide spectrum of diseases. The methods of the studies can be integrated. The methods further prove the increased risk of the population.

PO41

Development of a Photo-Genotoxicity Test Method Using the Reconstructed Skin Micronucleus Assay

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Abstract

To address a lack of suitable tools to screen novel ingredients in personal care products for mutagenic/clastogenic activity after solar light exposure, Cosmetics Europe and IFRA initiated a three-phase project to integrate established UVA/visible light photo activation techniques to the reconstructed skin micronucleus (RSMN) assay. An objective of this 3-phase project will be to generate limited data to demonstrate that pro-mutagens requiring photo activation can be detected and an evaluation can be made whether the new methodology shows merit. The program will proceed with the following milestones:

- Phase 1: Establish an appropriate photo irradiation schedule to identify a maximum tolerated UVA/visible light exposure compatible with the repeat dose regimen of the 72-hour RSMN dose protocol. A targeted outcome is the identification of a UVA/visible light exposure resulting in no significant

increases in micronuclei, and in no less than 90% viability relative to the dark exposure controls.

- Phase 2: Identify a known photo-genotoxic substance to be used as a test system positive control, and subsequently conduct proof of concept trials of the test methodology. The ideal chemical will be non-mutagenic in the absence of photo activation, but should be known to induce micronuclei in replicating mammalian cells after photo irradiation. Accordingly, 8-methoxypsoralen (8-MOP), which acts by DNA adduct formation and DNA intercalation after UVA exposure will be tested. Standard RSMN positive and negative controls will be tested in parallel in the absence of UVA/visible light to ensure standard test system performance. Protocol procedures may be optimized prior to proceeding to Phase 3.
- Phase 3: Expand proof of concept testing to include additional photo-genotoxins, as well as mutagens that are not activated by UVA/visible light; the latter "negative control" to determine whether differences in micronuclei induction can be measured when comparing treated tissues in the presence and absence of UVA/visible light exposure. Standard RSMN positive and negative controls will be tested in parallel in the absence of UVA/visible light to ensure standard test system performance.

The successful methodology is envisioned to be formally evaluated and submitted for regulatory acceptance for hazard identification purposes.

PO42

Animal-Free Safety Assessment of Cosmetics: a global education and training program

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Abstract

Great strides have been made in the ability to use data from non-animal assessment methods to establish safety of personal care products. However, a lack of familiarity with these data and processes inhibits uptake, both for product developers and for those who must assess the safety of those products. To increase confidence and enable local capacity, a large collaboration has been established to create a program to familiarize global stakeholders with using new

types of information and approaches for evaluating safety of cosmetic products and ingredients. The first step in such a risk assessment is to understand the product, its use, and the safety question that needs to be addressed. The assessment is exposure-led, so that consumer exposure is the first consideration. If exposure suggests there is a possibility of risk, hazard assessment is carried out, followed by refinement of the exposure estimate if necessary. Finally, all this information is combined in the risk assessment which includes estimates of uncertainty. The AFSA Cosmetics Education and Training Program covers the entire risk assessment process in 8 modules: Problem Formulation, Consumer Exposure, In silico Tools, Exposure-based waiving, Internal Exposure, In vitro Data Synthesis, and the Overall Risk Assessment. A ninth module covers the regulatory landscape of chemicals and consumer products. The program is built on established principles and processes and illustrated throughout with case examples from our members' experience. This presentation will provide an update on the development of this program.

PO43

Refinement of the toxicokinetic assessment of bisphenols in 2D in vitro liver models

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Abstract

Appropriate toxicological risk assessment of chemicals is an inevitable goal if we want to preserve a healthy environment for humans and wildlife. *In vitro* analyses are an approach to determine concentration-response relationships of chemicals in cell-based models.

One basic problem of many *in vitro* studies is that *in vitro* toxicity concentration-response relationships are still often based on the nominal concentration of a given toxicant. However, it is known that the added chemical is partitioning within the *in vitro* system. The substance can bind, for example, to serum proteins and lipids, can sorb to plastic equipment, evaporate, transform, or metabolize. All these processes can change the effective concentration of the added chemical during exposure time significantly. Using the nominal concentration for risk assessment can therefore lead to an underestimation of the risk. Determining or

modeling the effective concentration of a substance in the cells or the free available concentration (directly correlated with the concentration in the cells) is more appropriate for *in vitro-in vivo* extrapolation.

The restrain in applying more precise *in vitro* toxicokinetic approaches can likely be attributed to their complexity, leading to additional experimental effort, higher expenses, and need for additional expertise. We face this challenge in our work and improve our *in vitro* 2D model based on HepG2 cells. We will introduce and describe optimized experimental and computational approaches applicable for high-throughput analyses on a case study exposing HepG2 cells to bisphenols (A, F, S). These approaches will provide an affordable way for better *in vitro-in vivo* extrapolation.

PO44

Adaptation of the Alginate Immobilization of Metabolic Enzymes Platform to a 3D Bioprinting Approach for Metabolism-based High-throughput Screening

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Abstract

Acceptance and use of *in vitro* data for hazard identification, prioritization, and risk evaluation is limited in part by uncertainties associated with xenobiotic metabolism. Most *in vitro* systems lack the biotransformation capabilities of intact *in vivo* systems. This raises the possibility of overestimating the hazard of compounds that are rapidly bioinactivated *in vivo* or underestimating the hazard of those that are transformed to more active metabolites. The Alginate Immobilization of Metabolic Enzymes (AIME) is a lid-based method for retrofitting bioassays with metabolic competence. However, accessibility and throughput of the AIME platform is limited by proprietary custom materials and a complex workflow. The objective of this study was to address limitations of the lid-based AIME method through the incorporation of automated 3D bioprinting, with the goal of depositing S9-encapsulated microspheres directly into standard 384-well plates and including requisite cofactors for phase I and II hepatic metabolism.

Measurement of phase I cytochrome P450 (CYP) 3A4 enzyme activity using both the lid-based and automated bioprinting platforms revealed that the two approaches are comparable in terms of inter-well variation and signal strength. Further, the activity of a panel of additional phase I CYP enzymes (CYP1A2, CYP2B6, and CYP2C9) and phase II glucuronidation was measured using the automated bioprinting method. Further refinement of this assay system has the potential to expand high throughput screening capabilities in a robust, accessible way to incorporate *in vitro* xenobiotic metabolic competence. These views do not necessarily reflect the views or policies of the U.S. EPA.

PO45

Importance of Descriptor Selection in Classification Models: A Case Study for Comedogenicity

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Abstract

Selecting both significant and the minimum number of descriptors for the model is an important process in classification models. The selected descriptors are expected to be simple and interpretable, as well as providing good predictive ability. Due to the very large size of descriptor pools, descriptor selection method becomes a crucial importance. While fewer descriptors in a model provide more interpretable and more practical models, excess number of variables may lead to overfitting in a model. Although searching for the all-possible subsets of descriptors is the best approach to develop the best model, it is impractical because of time and energy constraints originated from a high number of candidate descriptors.

In this study, we applied and compared the correlation-based (via *CfsSubsetEval*) and learner-based (via *WrapperSubsetEval* with *J48 classifier*) feature selection in WEKA as the descriptor selection method on two different descriptor groups (alvaDesc and Mold2). The developed models via Random Forest successfully predicted the comedogenicity of the set of compounds from various chemical classes under study. While models' fit and predictivity were almost unchanged, the number of descriptors was reduced by 60-70% by the means of learner-based

descriptor selection that leading to simpler and more interpretable models. Futhermore, the models built via the learner-based method are more valid than the models built via the correlation-based for both two descriptor groups.

This case study showed the importance of descriptor selection method in terms of Topliss ratio that was related to the number of descriptors in the model.

PO46

NanoAmes™: An Ultra-miniaturized Format of Ames Test

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Abstract

The assessment of mutagenicity potential of complex mixtures containing unidentified substances is challenging. Today, the most widely used test to identify mutagenic substances is the Ames test, which is associated with the lowest calculated limit of biological detection (LOBD) values. Miniaturized formats of the Ames test were reported to be more sensitive than the standard regulatory Ames assay but the LOBDs are still inadequate to cover regulatory and safety requirements for complex mixtures, which possibly contain mutagenic agents at very low, but health-relevant concentrations.

An ultra-miniaturized agar-based Ames method, the NanoAmes™, with promisingly improved detection capacity, was developed and evaluated with a set of 11 mutagenic compounds, selected to compare the dose response curves on five bacterial strains (in presence and absence of metabolic activation) to those of MicroAmes assays run in parallel. Mutagenicity effect was consistently detected at a significant lower sample requirement and with more favorable lowest effective concentration values. Aiming to address the determination of the LOBD, a new statistical approach is proposed to analyze Ames dose response curves to extract the minimum detectable value (MDV). According to the current study, the NanoAmes™ performed better in terms of lower detectability capacity (in average

68× compared to the MicroAmes). Further analyses applying complex mixtures samples with spiking analysis at low dose of mutagenic compounds will confirm the application of this promising tool for mutagenicity assessment of unknown compounds potentially present in mixtures.

PO47

Development of a 5α-reductase High-throughput Screening Assay for Androgen Steroidogenesis

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Abstract

The US EPA employs high-throughput screening assays to identify environmental chemicals that may pose a risk to human health. Many assays are utilized by the Endocrine Disruptor Screening Program to evaluate effects on estrogen, androgen, and thyroid endocrine pathways. Altered androgen hormone biosynthesis contribute to endocrine disruption that may result in impaired reproductive and sexual development. Steroid 5α-reductase enzymes catalyze the conversion of testosterone into the more potent androgen 5α-dihydrotestosterone. Type 2 5α-reductase enzyme (*SRD5A2*) deficiency is associated with decreased virilization in males and presents an important mode-of-action when evaluating environmental chemical exposure. The objective of this study was to develop a high-throughput assay for screening inhibition of human SRD5A2. NanoBRET Target Engagement assay technology was used to evaluate modulation of testosterone binding to SRD5A2 in a 384-well cell-based format. Michaelis-Menten kinetics of the SRD5A2-NanoLuc fusion protein demonstrated normal conversion of testosterone to 5α-dihydrotestosterone (Km: 434 nM). Initial evaluation with 5α-reductase reference inhibitors dutasteride, finasteride, and epristeride revealed significant gain-of-signal. Screening of 1803 blinded ToxCast chemicals identified 91 chemicals with bioactivity hit counts. Additional filtering for assay-specific cytotoxicity and autofluorescence resulted in 24

chemicals with putative bioactivity toward SRD5A2. Overall, the NanoBRET assay technology demonstrated high precision (rCV: 5.2%), modest dynamic range (S/B: 1.41 FC), and marginal assay quality (rZ': 0.13). Finasteride was the most potent compound identified in the screen, suggesting sufficient sensitivity for identifying potent inhibitors of enzyme function. *The views expressed do not reflect the views or policies of the U.S. EPA.*

PO48

Adaptation of a Human Pluripotent Stem Cell Assay for Developmental Toxicity Screening

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Abstract

The US EPA is committed to developing non-animal, new approach methods to detect chemical risks to susceptible populations, including pregnant women. Additional coverage for cellular processes associated with human development in the ToxCast and Tox21 assay portfolio could enhance identification and prioritization of potential developmental toxicants. The objective of this study was to adapt a human pluripotent stem cell-based high-throughput screening assay to assess potential teratogens. For this assay, a transgenic human pluripotent stem cell line (RUES2-GLR) containing three fluorescent germ layer reporter biomarkers, SOX2–mCitrine (ectoderm/pluripotency), BRA–mCerulean (mesoderm), and SOX17–tdTomato (endoderm), was used to assess perturbations to directed stem cell differentiation toward definitive endoderm using high-content image analysis. After two days of chemical exposure, cytotoxicity was measured via nuclear stain and the percentage of SOX17+ cells was obtained to examine developmental toxicity. Initial testing was done on four reference teratogens (all-trans retinoic acid, lenalidomide, pomalidomide, thalidomide) and four non-teratogens (aspirin, caffeine, folic acid, saccharin). Teratogens demonstrated IC_{50} values of 0.13, 0.15, 0.047, and 1.4µm respectively for Sox-17 expression, while non-teratogens had no effect on biomarker expression. For further evaluation, a training set of 58 chemicals was screened using the assay

and revealed a balanced accuracy >70%. This study successfully adapted a human stem cell-based developmental toxicity screening assay to assess chemical perturbations during endoderm differentiation with the potential capability to evaluate disruptions during differentiation to all three germ layer lineages. This abstract does not necessarily reflect EPA policy, nor endorse or recommend any products mentioned.

PO49

Abstract Sifter: A literature informatics tool for computational toxicology

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Abstract

The biomedical literature contains an abundance of information about the activity of chemicals in biological systems, including environmental toxicants of interest to the EPA. The goal of literature informatics research at the EPA's Center for Computational Toxicology and Exposure is to use the literature more effectively in computational toxicology. To this end, we have developed a novel approach to article retrieval in our Abstract Sifter application. The Abstract Sifter is a document retrieval tool that integrates the richness of PubMed with the powerful data-handling capabilities of Microsoft Excel. Results from searches are imported directly into an Excel sheet where the end-user can then use a novel "sifting" methodology for quick, agile relevance ranking of articles. The tool also enables article triage capabilities through easy tagging and noting functionality. The Abstract Sifter can also provide a high-level view of a corpus of literature for a defined set of chemicals of interest. This "landscape" view helps researchers assess the volume of literature in any given subject area to help with project scoping and chemical ranking and prioritization. Queries developed from the OECD Adverse Outcome Pathway (AOP) project connect key events in AOPs to the literature for chemicals on the Landscape sheet, offering evidence for inferring and investigating a chemical's mechanisms of action. The Excel format of the tool provides ease of use and facilitates collaboration. A new feature that allows mapping of ontologies to a corpus will be introduced. *This abstract does not necessarily represent U.S. EPA policy.*

PO50

Compilation and Characterization of a Human Skin Sensitization Data Set

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Abstract

Appropriate evaluation of new approach methodologies (NAMs) requires reference data for assessing a NAM's ability to predict an outcome of interest. Human data would provide the most relevant basis for such comparisons, but they are rarely available due to obvious ethical issues associated with toxicology testing in humans. One exception is data from skin sensitization tests that are routinely conducted using a wide range of materials. For this project, we collected data from 2277 human predictive patch tests conducted under two protocols, the human repeat insult patch test and the human maximization test. Data were collected from more than 1700 publications. We recorded protocol elements and positive or negative outcomes, calculated traditional and non-traditional dose metrics, and developed a scoring system to evaluate each test for reliability. Test information was considered adequate for use if 1) dose metrics were reported or calculable, 2) the primary report, test substance, and test type were identified, and 3) positive responses and the total number of subjects tested were reported. The resulting database, which contains information for 1366 unique substances, was characterized for physicochemical properties, chemical structure categories, and protein binding mechanisms. The complete database is publicly available on the NTP Integrated Chemical Environment website to serve as a resource for the development and evaluation of NAMs for skin sensitization testing. This project was funded with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C. The views expressed above do not necessarily represent the official positions of any federal agency.

PO51

Skin Sensitization Testing of Mixtures Without Animals

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Abstract

Skin sensitization testing is a regulatory requirement for safety evaluations of pesticides in multiple countries. Globally harmonized test guidelines that include in chemico and in vitro methods reduce animal use, but no single assay is recommended as a complete replacement for animal tests. Defined approaches (DAs) that integrate data from multiple non-animal methods are accepted, but these DAs were evaluated with mono-constituent substances. This may limit their applicability to multi-constituent substances such as pesticides. This analysis evaluated rule-based DAs for hazard and/or potency categorization of skin sensitization for agrochemical formulations. To obtain data for the analysis, we tested 27 formulations using the direct peptide reactivity assay (DPRA), the KeratinoSens™ assay, and the human cell line activation test (h-CLAT). Balanced accuracy (BA) of the DAs for predicting skin sensitization hazard in vivo ranged from 56% to 73%. The best performing DA for GHS potency classification had a correct classification rate of 52%. KeratinoSens had the highest performance for predicting in vivo hazard outcomes (BA = 81% vs. 62% for DPRA and 56% for h-CLAT), which was higher than any of the DAs. These results demonstrate that non-animal test methods have utility for evaluating the skin sensitization potential of agrochemical formulations. Further investigation will be required to determine whether DAs can outperform individual assays for predicting in vivo sensitization hazard of pesticide formulations in general. This project was funded with federal funds from the NIEHS, NIH under Contract Nos. HHSN273201500010C and HHSN273201400017C; Corteva funded the DPRA and KeratinoSens testing.

PO52

Building confidence in alternative methods through ICE

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Abstract

New approach methodologies (NAMs) are generally defined as non-animal methods or approaches using one or more in vitro or in silico methods to provide insight on chemical hazard. While scientific and policy advances have enabled adoption of some NAMs for specific applications, barriers remain to broader acceptance of NAMs for regulatory purposes, where animal-based testing paradigms remain the standard. The National Toxicology Program's Integrated Chemical Environment (ICE) addresses these barriers to build confidence in NAMs. ICE provides access to high-quality, curated, regulatory-relevant data and in silico predictions of chemical properties. ICE computational tools allow users to search for, visualize, and obtain context for these data. ICE data acquisition and curation processes are transparent and include citations to original data sources. Efforts are underway to apply controlled vocabularies during curation to increase interoperability of data. High-throughput screening assays from the ToxCast and Tox21 programs have been annotated to mechanistic targets and modes of action to provide biological context for assay results. Curated data from these assays can easily be viewed in concentration-response format using the Curve Surfer tool. Other computational tools available in ICE allow users to run physiologically based pharmacokinetics and in vitro to in vivo extrapolation models and to search for structurally similar chemicals. These tools are designed to be accessed by diverse end-users through simple user interfaces. This project was funded with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.

PO53

The Impact of Study Inclusion Criteria on the Determination of Point of Departure Methods in the Carcinogenicity Potency (CPDB) Database

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Abstract

The carcinogenicity potency (CPDB) database is instrumental in consolidating testing results in the literature and the National Toxicology Program (US NIH NIEHS). Efforts to update the database have recently been reported to include <70 new substances with possible benchmark doses. The original CPDB database offers multiple studies per substance, multiple tests per study, and numerous site/effects per dose group. The point of departure (POD) at a substance level is extracted from this information. The TD50 (dose which 50% of the subjects are expected to have tumors) was used as the POD for the original CPDB database. Although the database has been used for hazard identification for carcinogenicity, the applicability of TD50s for potency assessment is controversial. Herein, we demonstrate how study inclusion criteria to select quality data for dose-response modeling affects the results of BMDL/BMD calculations. Dose response modeling results can be performed at different levels (i.e., study, test, dose group, and tumors at specific sites) and with common approaches for obtaining an aggregated BMDL. Our study inclusion criteria specify species, exposure route, duration, number of dose groups (e.g., single-dose studies excluded), tumor descriptions (e.g., no mixed tumors/sites), and expert opinions (e.g., no negative observations or non-human related findings). A modeling set was prepared by querying the database for studies with these criteria, and both PROAST and BMDS packages were applied to determine POD values. The impact of inclusion criteria, method used to estimate the reported POD, and recommendations for future analyses of new substances are discussed.

PO54

Computational Support of Pharmacokinetic Models and In Vitro to In Vivo Extrapolation

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Abstract

Pharmacokinetic models and in vitro to in vivo extrapolation (IVIVE) allow researchers to predict the in vivo distribution and bioactivity of toxicants by quantitatively relating in vitro results to in vivo systems. By translating in vitro experimental concentrations into relevant in vivo doses, these nonanimal alternative test methods have the potential to inform regulatory decisions by providing a margin of exposure context. Additionally, pharmacokinetic models can inform hazard screening prioritization by estimating tissue-level concentrations to characterize the distribution of bioactive compounds. Quantitative structure-activity/property relationship modeling tools such as the OPEn structure-activity/property Relationship App have broadened the applicability of physiologically based pharmacokinetic (PBPK) and IVIVE analyses by extending the range of potential chemical analytes to all substances with defined structures. To further expand the transparency and accessibility of PBPK and IVIVE models, we have developed open-access computational tools to facilitate customized simulations and analysis. The National Toxicology Program's Integrated Chemical Environment includes graphical user interface tools for both PBPK modeling and IVIVE that allow users to specify the model complexity, species of interest, exposure route, dosing regimen, simulation time, and any relevant in vitro assay data. These tools provide a well-documented, easy-to-understand platform to apply PBPK and IVIVE modeling to chemicals of interest. Future applications of these tools and techniques may provide valuable insight for regulatory decision making. This project was funded with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.

PO55

Genome profile of resistance to P450-activated carcinogens and pharmaceuticals using humanized yeast libraries

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Abstract

Many human carcinogens and pharmaceuticals require activation by cytochrome P450 (CYP) enzymes to become potent genotoxins. The budding yeast deletion library, composed of over 5,000 strains individually deleted for a unique gene, has been fundamental in identifying eukaryotic genes that confer

resistance to multiple drugs and carcinogens, many of which are evolutionary conserved in mammalian organisms. Since many potent carcinogens require CYP activation, we have introduced expression vectors containing CYP1A2 or CYP1A2 and NAT2 into the yeast deletion library. We profiled the yeast genome for resistance to aflatoxin B1 and heterocyclic aromatic amines when CYP1A2 was expressed and when it was absent. While only one aflatoxin resistance gene was identified from the original yeast library, 86 resistance genes were identified in the library expressing CYP1A2. The gene ontology groups represented included DNA repair, protein degradation, and DNA damage tolerance. To further define the role of DNA repair, we have created smaller humanized libraries, containing 160 strains. Additional DNA repair genes that we identified included those involved in mismatch repair, DNA replication fork stability, and DNA double-strand end resection. Additional studies are currently in progress to determine whether orthologous genes in mammalian cells function in conferring carcinogen resistance. These studies should expedite the identification genes that confer resistance to multiple CYP-activated compounds.

PO56

Scale-up considerations for new *in vitro* toxicology approach methodologies

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Abstract

For many years *in vivo* testing has been the gold standard for toxicology. Currently, the field is transitioning towards the use of new approach methodologies (NAMs). In this regard, the supplier industry has been diligent in the fostering of NAMs and there are many cases of synergy with other stakeholders towards the 3Rs principle. Despite these advances, the wide adoption of alternative *in vitro* methods for toxicology testing is a current challenge. The effective industrial manufacturing of NAMs is critical to accelerate the *in vivo* to *in vitro* transition. To efficiently translate a NAM into regulatory, commercial, and technical requirements for large-scale manufacturing, the early engagement with a mature industrial partner is encouraged. From our 60-years of experience as plastic and membrane OEM of commercial off-the-shelf and customized NAMs, we would like to summarize some key points that we believe

will help scientists to translate their ideas in line with financial and regulatory specifications. For instance, the upscaling of a new device implies an attentive evaluation of the required times and costs. Consequently, the scientific relevance, potential market size, scalability, reproducibility, and high-throughput potential must be considered in advance. In addition, elements such as material and tolerance levels, quality control, homogeneity, regulatory strategy, environmental conditions, packaging, appearance, functionality, ergonomics, ecology, shelf life, sterility, labelling and transport are of extreme importance. The supplier industry is open to nurture NAMs and it is committed to help reduce, replace, and refine the use of animals in toxicology testing, while advancing human benefit.

PO57

Comparison of the expression of SARS-CoV-2 receptors in *in vitro* skin and lung models

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Abstract

The outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused the pandemic associated with the severe acute pulmonary disease named COVID-19 (coronavirus disease 2019). The exact pathogenesis of severe COVID-19 remains unclear, but it typically involves a hyperinflammatory response following viral infection and induces significant damage in the respiratory tract.

The recommended hygiene procedures for the fight against COVID-19 include repeated handwashing and frequent use of hand sanitizer that can disrupt the skin barrier integrity. Loss of skin barrier integrity represents a potential transmission route for SARS-CoV-2 through the skin.

The entry of SARS-CoV-2 in host cells depends on the availability of virus receptors and entry cofactors on the surface of host cells. The most important receptors identified so far are the Angiotensin Converting Enzyme 2 (ACE2) receptor, the transmembrane serine protease 2 (TMPRSS2), and the Neuropilin-1 (NRP-1), all three receptors are under the control of Androgen Receptor (AR).

In this talk, we will present the constitutive expression of SARS-CoV-2 key receptors; ACE2, TMPRSS2, NRP1 and AR in primary culture of human epidermal keratinocytes and human dermal fibroblasts, in 3D-reconstructed human epidermis model and in human skin biopsies. Comparison will be made with Calu-3 cell line and 3D-EpiAirway model, representing lung models. Moreover, effect of stimulation with lipopolysaccharide (inflammatory agent) on the modulation of mRNA expression of cytokines markers and SARS-CoV-2 receptors will be measured and compared in the *in vitro* skin and lung models.

PO58

Assessment of interaction toxicity of per-and polyfluoroalkyl substances (PFAS) mixtures using in vitro systems

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Abstract

In this study, the combined effects of per- and polyfluoroalkyl substances (PFAS) was investigated by treating human liver cells (HepG2) with various concentrations of perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), perfluorodecanoic acid (PFDA), perfluorononanoic acid (PFNA), and perfluorohexanoic acid (PFHxS) individually or in binary combinations (PFOS + PFOA, PFOS + PFDA, PFOS + PFNA, PFOS + PFHxS, PFOA + PFDA, PFOA + PFNA, and PFOA + PFHxS) for 24 h using an orthogonal design. The individual and binary combination effects of PFAS on the cytotoxicity, intracellular reactive oxygen species (ROS) production, and glutathione (GSH) levels were determined by MTS assay, dichlorofluorescein diacetate assay, and GSH-Glo™ Glutathione assay, respectively. The results showed that exposure to PFOA, PFOS, PFDA, PFNA, and PFHxS individually and in binary combinations caused concentration-dependent cytotoxicity to HepG2 cells. Also, intracellular ROS production was not significantly induced in both the individual and co-treatment groups, indicating that ROS production may not be likely influencing the combined cytotoxicity of PFAS to HepG2 cells. However, the depletion of the intracellular glutathione levels was correlated with cytotoxicity. Moreover, the factorial analysis results showed no significant interactive effects between PFOS + PFOA, PFOS + PFDA, PFOS +

PFNA, PFOS + PFHxS, PFOA + PFDA, PFOA + PFNA, and PFOA + PFHxS. Taken together, the results showed that both individual and combined PFAS could induce concentration-dependent cytotoxicity and depletion of GSH levels, but could not induce significant increases in ROS production at the concentration range tested. Overall, these results provided toxicological data on the combined effects of mixed PFAS that may help to better assess their health risk.

PO59

An Example Demonstration of In Silico Skin Suite

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Abstract

Comprehensive assessment of chemical toxicity instigated by dermal contact requires addressing exposure, i.e., permeability of a chemical through the skin, as well as chemical reactivity that may lead to irritation or subsequent sensitization via well-known induction/elicitation immune response mechanisms. The ChemTunes.ToxGPS® In Silico Skin Suite consists of rule-based profilers and QSAR models for stepwise assessment of key dermal toxicity endpoints. First, the Skin Permeability Profiler, developed using a training set of human in vitro Kp data curated by domain experts, classifies chemicals according to their skin permeability potential into low, medium and high categories by applying Chemical Subgraphs and Reactions Markup Language (CSRML) rules representing both structural features (ToxPrints) and physical/chemical property information within a single object. Next, skin irritation is evaluated in terms of a QSAR model classifying the chemicals into two categories (irritants, non-irritants) defined on the basis of principal irritation index (PII) values. Skin sensitization hazard (sensitizer, non-sensitizer) and potency (GSH categories) are then assessed based on structural alerts and mode-of-action QSAR models. Finally, a quantitative weight-of-evidence approach is applied to combine various evidence sources and estimate the prediction uncertainty. This poster demonstrates the In Silico Skin Suite as implemented in ChemTunes.ToxGPS® by demonstrating its application to a cosmetics substance.

PO60

OrbiTox – A Translational Discovery Platform for Concerted View and Analysis of Multi-domain Data

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Abstract

A typical toxicological inquire relies on data housed in various resources such as DSSTox, PubChem, ChEMBL, DrugBank, TOXNET and more. An opportunity to derive knowledge from connectivity among, and concerted view of, these data is missed because they exist in silos, in different formats, and require different filtering and searching mechanisms. We present OrbiTox - an interactive 3D visualization and analysis platform for varied scientific big data with special emphasis on predictive toxicology.

OrbiTox is an immersive environment with tens of millions of experimental and modelled data-points, and data gap-filling predictive models. To enable translational discovery via a collective view of data from different domains and their interrelationships, OrbiTox projects high-dimensional multi-domain data in concentric 3D layers. The Chemistry layer contains ~800,000 substances with structure, Tox21 qHTS, mutagenicity, and carcinogenicity data. Approximately 25,000 fully annotated human genes and 2000 pathways with functional annotation are populated in the Gene and Pathway layers. The Species layer contains data such as genus, family, class, life span, average weight, etc., on species on which toxicity tests have been done.

Additionally, for data gap filling purposes, OrbiTox offers 41 QSAR models for Tox21 assays and models for predicting bacterial mutagenicity with and without metabolic activation in OECD-compliant strains. These models provide chemistry-backed reasoning for prediction based on *Saagar* - our recently published set of new 834 molecular features (Sedykh et al. 2021).

This unique arrangement of experimental and predicted data and connections made across data domains enables knowledge extraction previously very onerous.

PO61

Development of an Internal Threshold of Toxicological Concern (iTTC) through PBPK modeling

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Abstract

A multi-stakeholder collaboration is working towards extending the Threshold of Toxicological Concern (TTC) concept which can be applied to internal concentrations rather than external oral exposures. This refinement of TTC based on plasma concentration, referred to as internal TTC (iTTC), converts the chemical-specific external NOAELs (mg/kg/day) in a TTC database to an internal exposure using Physiologically Based Pharmacokinetic (PBPK) modeling. The current presentation describes the PBPK modeling work that was done using Population Life-course Exposure to Health Effects Model (PLETHEM), updated to run batch mode, to quantitatively identify ADME relationships. An initial literature review for existing *in vivo* and *in vitro* pharmacokinetic (PK) data was done for the more than 1200 chemicals that comprise the iTTC database. The literature review results were used to parametrize and evaluate PBPK model simulations and refine the initial chemical list (~300) guide where additional *in vitro* metabolism data was necessary. Chemicals with *in vivo* PK and *in vitro* metabolism data were used to evaluate PBPK model simulations. Multiple simulation conditions, including, oral absorption, partition coefficient algorithm, and plasma protein binding were

evaluated for their impact on simulation output. The modeling workflow has performed well using Cmax and AUC predictions being within 4-fold of the experimental data for 86% and 70% of the simulations, respectively. Currently permeability and metabolism data are being collected using Caco-2 cells (~300 chemicals) and hepatocyte suspensions (~200 chemicals). Once confidence is established for chemicals with PK data it can be reapplied to chemicals that are data poor.

PO62

Estimating human skin sensitization potential with an assembly of human and animal QSAR models

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Abstract

Estimating the potency values for contact allergens is of considerable importance when determining proper risk labels. The computational approaches for assessing skin sensitization are gaining wider regulatory acceptance. This presentation will present a workflow to assess the quantitative risk of skin sensitization for humans using QSAR models developed from animal and human data. An assembly of classification models for LLNA and Guinea Pig Maximization Test, Buehler test, Human Maximization Test, and Human repeat Insult Patch Test, as well as three categorical LLNA models, are used. The workflow consists of three steps and is very intuitive and highly interpretable. To validate this approach, the initial set of known human sensitizers and non-sensitizers was assembled. After removing the chemicals already present in the learning sets of the used QSAR models and additional data curation, the final external set consists of 19 compounds, 14 sensitizers, and five non-sensitizers. This set was predicted with coverage 94.74%; sensitivity 92.31%; specificity 80.00%; concordance 88.89%, positive accuracy 92.31%, and negative accuracy 80.00%. Thus, we suggest a highly predictive workflow to assess the risk and potency of human skin sensitizers.

PO63

Using adverse outcome pathways to categorize new alternative methods for predicting acute oral systemic toxicity

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Abstract

Regulatory agencies around the world have committed to reducing or eliminating animal testing for establishing chemical safety. Replacements include both in silico and non-animal approaches such as the Collaborative Acute Toxicity Modeling Suite (CATMoS). Adverse outcome pathways (AOPs) provide a mechanistic framework for connecting upstream molecular/cellular targets, evaluated by in vitro toxicity tests and in silico predictions, to apical adverse outcomes (AO) such as the LD50 endpoint used in traditional oral acute toxicity (AT) studies. This study categorizes CATMoS chemicals according to the mechanism by which the chemical causes toxicity, as a step towards identifying a limited set of upstream mechanisms within AOPs that terminate in AT as the AO. Association rule mining was used to link pathways, phenotypes, diseases, and apical endpoints retrieved from the Comparative Toxicogenomics Database (CTD) and ChemID*plus* based on the co-occurrence of the endpoints across chemical activity profiles. We then used a random walk procedure to coalesce highly connected pathways and phenotypes (referred to collectively as CTD mechanisms) into communities. Out of 260 CTD mechanisms manually mapped to known AT mechanisms, just 16 CTD mechanisms occurred in the communities. 31% of acutely toxic CATMoS chemicals with CTD Pathway data associate with at least one of the 16 AT-Community Mechanisms. We are currently using CTD mechanisms linked to the remaining 69% to expand the coverage. The final AOP inventory will be used to identify AOP development needs, check coverage of mechanistic assays in the AT chemical space and prioritize assay development for acute systemic toxicity screening.

PO64

Air-Liquid Interface Re-Submersion: Alternatively Regulates Stress-Responsive Signaling, Enhances Cytokine Secretion, and Increases Barrier Permeability in Primary Human Bronchial Epithelial Cells.

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Abstract

The *in vivo* functions of the bronchial epithelium can be recapitulated *in vitro* through the differentiation of primary human bronchial epithelial cells (pHBEC)

under air-liquid interface (ALI) culture conditions. pHBEC ALI models have rapidly gained popularity for *in vitro* chemical testing and research; however, there are practical challenges facing the delivery of many test agents to ALI cultures. These limitations have led to the common practice of suspending methodologically challenging test agents (e.g., particles and aerosols) in aqueous solution and dosing by re-submersion of differentiated ALI cultures. Given the physiological characteristics that develop during pHBEC ALI differentiation, we hypothesized that re-submersion alone would have a significant effect on common *in vitro* toxicity endpoints. To test our hypothesis, we re-submerged differentiated organotypic pHBEC/lung fibroblast ALI co-cultures in basal growth medium and examined global gene expression, epithelial barrier integrity, and pro-inflammatory cytokine secretion. Re-submersion resulted in the significant alternative regulation of 4038 and 7499 genes in pHBEC at 6 and 24 hours, respectively, with many of the most dysregulated genes being involved in stress-responsive cell signaling and inflammation. These transcriptional changes were complemented by significant increases the secretion of the pro-inflammatory cytokines IL-8, IL-6, IL-1β, and TNFα. We also observed a progressive decrease in trans-epithelial electrical resistance and increase in small molecule permeability. Cumulatively, our findings indicate that the use of re-submersion approaches to test agent dosing in ALI cultures is likely to be a substantial confounding factor in chemical testing that requires further characterization. Abstract does not reflect EPA policy.

PO65

Increasing Reproducibility Using FBS-Free Media: A Case Study in Transitioning A549 Cells

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Abstract

Replacing the use of fetal bovine serum (FBS) in cell culture media bolsters the reproducibility of *in vitro* research and overcomes the ethical and legal challenges

associated with its use. Increasingly, scientists are focusing on replacing the use of FBS as a supplement in cell culture media with animal-component-free media.

Using A549 cells—an immortalized human epithelial alveolar-like cell line commonly used in respiratory research—as a case study, we demonstrate the process of transitioning cells cultured in medium containing FBS to commercially-available media without FBS. To determine whether the transition was successful, cellular morphology and functionality were assessed by imaging (scanning electron microscopy); calculating cell doubling time, cytokine release (Bio-Plex), and cell viability (Alamar blue assay); monitoring the expression of relevant genes; and determining surfactant production (surfactant droplet test). Our results show that, while success varies based on the transition process and type of media, animal derived components can be replaced in the culture of A549 cells. Because FBS-free media can replicate the phenotype that A549 cells demonstrate in FBS-supplemented medium or can demonstrate characteristics of more normal alveolar epithelial cells, the media chosen should be determined by the study objective. This case study can be used as a guide to transition other cell types to FBS-free media.

PO66

Characterization of TiO_2 cytotoxic behavior on HaCaT and A549 by three different viability assays

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Abstract

The purpose of this work is to evaluate the potential cytotoxic effect of microsized and nanosized (21 nm) TiO_2 on different cell lines with three different viability assays.

Mean hydrodynamic diameter of TiO2 particles were determined by dynamic light scattering using a Malvern Zetasizer ZS. Particles (1.0 mg/ml) were appropriately diluted in phosphate buffered saline (PBS, pH 7.4), in PBS containing bovine

serum albumin (BSA) or fibrinogen (Fib) at 2.0 mg/ml and in DMEM 5% FBS, and incubated for 2h and 24 h at room temperature. Bradford assay quantified protein adsorption, whereas prothrombin (PT) and partial thromboplastin time (PTT) allow to evaluate effects on blood coagulation time. Cytotoxic effects of TiO2 particles on HaCaT and A549 were evaluated at different concentrations (from 0.78µg/ml to 100µ/ml) at 24 hours by the MTT, NRU and LDH methods.

Particle diameter, independently of the incubation time and size, decreased by the presence of proteins that might prevent from the formation of agglomerates. The amount of adsorbed protein is less in the case of microsized particles but increases with time and is greater in the case of Fib. Coagulation time is affected by the presence of nanosized TiO_2 that decrease PT and PTT, whereas microsized particles decrease PT and increase PTT. Cytotoxic behavior differs between micro and nano TiO_2 in the case of on HaCaT when cell viability is determined by LDH being the IC_{50} 14µg/ml. In the case of A549 cells, no differences in cell viability can be attributed to particle size.

PO67

In vitro-in vivo extrapolation (IVIVE) for neurodevelopment: Toxicokinetics and *in vitro* point of departure evaluation of putative developmental neurotoxicants

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Abstract

In vitro new approach methodologies (NAMs) are increasingly applied to screen for developmental neurotoxicity (DNT). While these NAMs hold many benefits over traditional *in vivo* DNT guideline studies, translating *in vitro* potency values to *in vivo* concentrations involves uncertainties, including data gaps regarding extrapolations of doses in the brain during critical windows of development, motivating development of a customized *in vitro-in vivo* extrapolation (IVIVE) approach. To provide an estimate of *in vivo* exposures required to elicit DNT-relevant bioactivity, here we have performed IVIVE using physiologically-based pharmacokinetic (PBPK) modeling—incorporating hepatic clearance and fraction unbound in blood to predict brain maximal concentrations (C_{max}) during different stages of neurodevelopment. This IVIVE approach was performed on 80 chemicals

with *in vitro* human toxicokinetic data that had been screened in DNT NAMs for bioactivity, including high content imaging of neural cell lines and a neural network formation assay. Across the chemicals tested, PBPK modeling revealed a variation in C_{max} of 6 orders of magnitude. Bioactivity information was incorporated to calculate an administered equivalent dose (AED)—this varied by 7 orders of magnitude, with the lowest AEDs typically found for the youngest age examined and for endpoints of cytotoxicity, network connectivity, and general network activity. By incorporating bioactivity and external exposure information, we provide metrics—bioactivity:exposure ratios—that can be used for decision-making regarding chemicals of concern for DNT. *The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.*

PO68

The terms "alternatives" and "alternatives to animals" should be confined to the history books.

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Abstract

"Alternative" is defined as "available as another possibility or choice" or "relating to activities that depart from or challenge traditional norms". An alternative to an animal is, therefore, a choice. Arguably, the second choice, starting with a steadily eroding view that the animal is the gold standard. This does not sound like the current state of science that has been developed over the past 50 years. New approaches to testing are based on scientific principles (e.g., AOPs and MIEs) which have to be demonstrated and justified, often against higher levels of scientific and regulatory scrutiny than the "alternative" animal test. We use human cells, tissues and increasingly complex advanced tissue and MPS models. These models utilise complex human biology, engineering solutions, computational evaluations in clearly identified, linked, processes. The animal is no longer needed in next generation risk and hazard assessment or efficacy evaluation, where the patient, the operator and the consumer with their particular needs and exposures are the focus. As an example, in respiratory toxicology, we use computational fluid dynamics to identify where particles may

be deposited in the lung to choose to evaluate toxicity in the upper or lower respiratory tract of human 3D models. We can change to human diseased derived 3D models for the vulnerable or patient, and reprogram the CFD for children. The alternative is an inbred healthy juvenile animal. The mainstream is now what was previously the alternative or new, it is now time to describe this as toxicology, metabolism and exposure science.

PO69

In silico Toxicity Prediction of Therapeutic Natural Products

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Abstract

During drug development, safety is always the most often and important hurdle including a variety of toxicities and adverse effects. *In silico* toxicity tools have been instrumental in screening lead molecules during development thus saving lot of time and efforts. The present study elucidates the *in silico* toxicity analysis of proven isolated therapeutic chemical constituents such as Rugosin D (isolated from *Rosa rugosa*), Oenothein B (*Epilobium angustifolium*), Geranin (*Nephelium lappaceum*), Agrimoniin (*Agrimonia pilosa ledeb*), Syringin (*Syringa vulgaris*), Peoniflorin (Paeonia suffructicosa), Boldine (*Peumus boldus*), and alstonine (Rauvolfia species). The *in silico* tools used for evaluation were Toxtree V3.1.0 and OECD QSAR Toolbox V 4.4, and webservers such as ProTox-II (version last updated in March 2020), DL-DILI Prediction Server, and the VEGA platform (version 1.1.5). The applications and webservers were used to predict a range of toxicity endpoints such as hepatotoxicity, carcinogenicity, mutagenicity, endocrine disruption, and skin sensitization. The compounds were assessed based on the consensus of results, and were labelled as positive or negative for a particular toxicity endpoint. Of all the 8 compounds, syringin, peoniflorin, boldine, and alstonine were belongs to Cramer Class III and remaining chemicals were belongs to Class I. All the compounds were negative for endocrine disruption and hepatotoxicity except geranin which was predicted to be hepatotoxic. In addition, boldine and alstonine have structural alert for genotoxic carcinogenicity and mutagenicity. Furthermore, peoniflorin, boldine, and alstonine have structural

alert of skin sensitization. These results can be useful in prioritizing the toxicity studies in further drug development.

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The American Society for Cellular and Computational Toxicology aims to provide an organized forum for discussion of cellular (*in vitro)* 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.

Individual and corporate memberships are available and support the activities of the society, including an annual meeting and a monthly webinar program, which is held in cooperation with ESTIV.

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 purpose
- 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 cosmetic, pharmaceutical, and chemical industry scientists and professionals.

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