ASCCT 12th Annual Meeting

Spotlight on NAMs: Elevating New Approaches in Risk Assessment

COMPLETE ABSTRACT BOOK

ASCCT

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Contents

CE Courses

CE1: Guidance for the Application of New Approach Methods (NAMs) for Hazard and Risk-Based Estimates Using the Exposure and Safety Estimation (EAS-E) Suite Platform

Alessandro Sangion[1], James Armitage[2], Li Li[3], Jessica Ponder[4], Jon Arnot[1,5]
[1]ARC Arnot Research and Consulting, Toronto, Canada. [2]AES Armitage Environmental Sciences, Ottawa, Canada. [3]University of Nevada Reno, Reno, USA. [4]Physicians Committee for Responsible Medicine, Washington, USA. [5]University of Toronto, Toronto, Canada

Abstract

New Approach Methods (NAMs) such as in vitro bioactivity assays are being developed to advance the capacity and pace of chemical assessment. Knowledge of chemical distribution in the test system is important to confidently apply the results for hazard and risk assessment. In Vitro Mass Balance Models (IV-MBM) provide quantitative predictions for chemical distribution in the bioassays and determine if significant volatilization, binding, or saturation has occurred. To apply the in vitro data in a risk-based context, in vitro-in vivo extrapolation (IVIVE) models that include toxicokinetic (TK) models, and related model input parameters are required. Aggregate exposure models for humans and ecological receptors, e.g., PROduction-To-EXposure High-Throughput (PROTEX-HT), can also be applied to estimate exposures. Combined these NAMs and models can estimate potential risk and help experimental design, i.e., dosing values. This Continuing Education describes a workflow to estimate Administered Equivalent Doses (AEDs) from nominal in vitro medium concentrations and steady-state blood concentrations (C_{SS}) using the IV-MBM and IVIVE models and then to compare these values with exposure predictions to calculate bioactivity-exposure ratios (BERs). The workflow is implemented in the free and publicly available Exposure And Safety Estimation (EAS-E) Suite online platform (www.eas-e-suite.com). The EAS-E Suite platform further facilitates the comparisons of different models and assumptions for calculating AEDs and BERs as illustrated for a set of case study chemicals spanning a wide range of partitioning properties and susceptibility to degradation. Recommendations for addressing key data gaps (uncertainties) and increasing confidence in the application of NAMs for regulatory decision-making are discussed.

First Presentation

Introduction and orientation to the Exposure And Safety Estimation (EAS-E) Suite platform.
Jon Arnot

Chemicals require safety and risk assessment and the necessary information is quite limited or non-existent for most chemicals. The freely accessible on-line Exposure And Safety Estimation (EAS-E) Suite platform (www.eas-e-suite.com) comprises curated databases of measured physical-chemical properties, environmental degradation half-lives, in vitro and in vivo toxicokinetic parameters, and production volumes for thousands of organic chemicals, as well as quantitative structure-activity relationships (QSARs) for predicting chemical information, if measured data are unavailable. EAS-E Suite includes various multi-media mass-balance (mechanistic) models for environmental fate (indoor and outdoor), in vitro and in vivo toxicokinetics, and exposure models for humans and a range of ecological

receptors (plants, invertebrates, fish, birds, and mammals). EAS-E Suite autoparameterizes the built-in models requiring a Chemical Abstract Service (CAS) number, name, or Simplified Molecular Input Line Entry System (SMILES) code for existing chemicals and a SMILES code for new chemicals. An overview of EAS-E Suite is presented.

Second Presentation

Estimating steady-state blood concentrations (C_{SS}) and Administered Equivalent Doses (AEDs) from cellular (in vitro) bioassays.
Alessandro Sangion

In vitro bioassays are being developed to advance the capacity and pace of chemical assessment. Knowledge of chemical distribution in in vitro test systems is imperative to confidently apply the results for hazard and risk assessment. In Vitro Mass Balance Models (IV-MBM) provide mechanistic insights and calculations for chemical distribution in in vitro bioassays and can indicate if significant volatilization, binding, or saturation is expected occurred to aid experimental design and data interpretation. IV-MBM, in vitro-in vivo extrapolation (IVIVE) models, and related model input parameters are required to apply the in vitro data in a risk-based context. This presentation includes an introduction to in vitro toxicokinetic and IVIVE modelling. A workflow to estimate steady-state blood concentrations (C_{SS}) and Administered Equivalent Doses (AEDs) from nominal in vitro medium concentrations using data and models built into the EAS-E Suite platform (www.eas-e-suite.com) is described and applied with a case example.

Third Presentation

Exposure predictions and calculating bioactivity-exposure ratios (BERs).
Jon Arnot

Exposure data are required for risk-based chemical evaluations; however, there is a paucity of relevant exposure measurements. Exposure models are necessary to address data gaps and integrate any available relevant measured information. The PROduction-To-EXposure High-Throughput (PROTEX-HT) model calculates external and internal aggregate chemical exposure to humans and a diverse range of agricultural and ecological receptors requiring only three model input parameters for data poor chemicals: SMILES notation (structure), production volume, and functional use category. PROTEX-HT consolidates data, models, and tools for simulating chemical emissions throughout their lifecycle, chemical fate in natural environments and food webs, chemical fate in indoor environments from use indoors and applications directly to the body, and toxicokinetic models. PROTEX-HT is implemented in EAS-E Suite. This presentation describes the PROTEX-HT model, its evaluation, and combines exposure predictions and in vitro data from Section 2 to calculate bioactivity-exposure ratios (BERs) as a case example.

Fourth Presentation

EAS-E Suite demonstration and participant trials

The instructors will provide specific case example demonstrations of the EAS-E Suite platform and participants will have the opportunity to try EAS-E Suite themselves. If participants are interested in

trying EAS-E Suite during the course, it is recommended they bring a laptop or tablet. They can register on-line before or during the course to gain access to the EAS-E Suite platform at www.eas-e-suite.com.

CE2: Open-access Data and Computational Tools to Investigate Chemical Bioactivity

Aswani Unnikrishnan, Victoria Hull, Alexandre Borrel, Kim T To, James T Auman
Inotiv, Research Triangle Park, USA

Abstract

To provide user-friendly access to high quality data and computational tools aimed at providing data from new approach methodologies (NAMs), the National Toxicology Program's Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) has developed the Integrated Chemical Environment (ICE) and other tools to support chemical safety assessment. This CE session will introduce the suite of tools included within ICE and three stand-alone tools, ChemMaps.com, the OPEn structure–activity/property Relationship App (OPERA) and the Defined Approaches for Skin Sensitization (DASS) App. Attendees will gain familiarity with how to search for and interpret data within the ICE platform, and the different ways to retrieve data for chemicals within the ICE database. This will be followed by a demonstration of multiple interoperable ICE tools that can be used to interactively explore and interpret ICE data, with examples of exploring high-throughput sequencing assays, predicted tissue concentrations, and exposure scenarios.   Attendees will learn to characterize and explore known and unknown chemicals by implementing OPERA models and interactively navigating chemical space with ChemMaps.com. The session concludes with a demonstration of the DASS App for predicting chemical hazard and potency using defined approaches for skin sensitization that have been internationally accepted by regulatory agencies. By the end of this CE session, attendees will understand how to use the capabilities available within NICEATM's computational platforms and tools, through demonstrations of tool functionality and applicability using cases studies to characterize chemical use, analyze available data, and identify potential hazards for their chemicals of interest.

First Presentation

Overview of NICEATM's Integrated Chemical Environment (ICE) with detailed insight into its Search and structural similarity prediction tools.
Aswani Unnikrishnan

The Integrated Chemical Environment (ICE) provides highly curated toxicologically relevant data and analytical tools for data interpretation and exploration. ICE's Search tool lets users query data for lists of chemicals and mixtures, yielding summary-level information and assay results mapped to mechanistic targets and modes of action. The latest ICE release introduced a beta Search query summary that provides summary visualizations to help users contextualize and interactively explore data. The Chemical Quest tool enables users to query chemicals or SMILES structures to identify structurally similar chemicals. Query results are ranked based on the similarity of the query chemical fingerprints with chemicals in the ICE database.

This presentation will provide an overview of ICE and demonstrate the use of Search and Chemical Quest tools through examples of chemical evaluation use cases, such as identifying information for data-

poor chemicals and comparing the bioactivity of chemicals across multiple toxicity endpoints of regulatory concern.

Second Presentation

Using the Integrated Chemical Environment (ICE) to access interoperable computational tools and inform chemical hazard
Victoria Hull

The interactive, interoperable tools in ICE enable users to interpret large amounts of toxicologically relevant data and implement complex models through a user-friendly interface. Within the Curve Surfer tool, users can visualize concentration-response curves from curated high-throughput screening data. They can also filter on criteria like mode of action, mechanistic target, and bioactivity, narrowing results to those that are most biologically relevant. The Physiologically-based Pharmacokinetic (PBPK) and In Vitro to In Vivo Extrapolation (IVIVE) tools implement models from the EPA's httk package to estimate chemical tissue concentrations over time and relate in vitro assay measurements to in vivo exposures, respectively. The Chemical Characterization tool can be used to explore physicochemical properties and compare potential chemical use cases through which consumers may be exposed. We will build upon case studies from the first presentation to demonstrate the use of these tools for exploring data and understanding potential chemical hazards.

Third Presentation

ChemMaps.com and OPERA: cheminformatics tools to navigating chemical space and characterize chemicals
Alexandre Borrel

With the increasing size and diversity of publicly accessible databases, particularly for environmental chemicals, navigating the chemical space has become crucial. To address this need, we have developed ChemMaps.com, a user-friendly web server inspired by Google Maps. ChemMaps.com facilitates easy exploration of the chemical space by employing complex projection techniques and molecular descriptors. In this new release, we have included all assay results available from the inter-agency ToxCast/Tox21 program. This presentation will demonstrate how ChemMaps.com can be utilized for read-across analysis, risk assessment, and the exploration of unknown chemicals. Additionally, we will introduce OPERA, an open-source suite of QSAR models that provides predictions for chemical properties of environmental significance. OPERA predictions can be downloaded from ICE and ChemMaps.com and can be generated for any new chemicals using the standalone version.

- ChemMaps.com: https://sandbox.ntp.niehs.nih.gov/chemmaps/
- OPERA: https://ntp.niehs.nih.gov/whatwestudy/niceatm/comptox/ct-opera/opera

Fourth Presentation

DASS App: A Web Application for Applying Defined Approaches for Skin Sensitization to Predict Hazard and Potency Categorization
Kim T. To

Skin sensitization is a critical regulatory toxicity endpoint associated with allergic contact dermatitis. Defined approaches for skin sensitization (DASS) have been developed to identify potential skin sensitizers by integrating non-animal test methods that represent key events in the skin sensitization adverse outcome pathway. We developed the DASS App, an open-source web application, to facilitate user application of four defined approaches that have been accepted by the OECD or U.S. Environmental Protection Agency. The DASS App enables users to implement non-animal approaches to evaluate chemical skin sensitization without the need for additional software or computational expertise. The app supports upload and analysis of user-provided data, includes steps to identify inconsistencies and formatting issues, and provides hazard predictions in a downloadable format. The DASS App is available on the National Toxicology Program website at https://ntp.niehs.nih.gov/go/40498.

This presentation will provide background information about the DAs, followed by a demo of the web application.

CE3: Putting Theory Into Practice: Using *in Vitro* and Computational New Approach Methodologies (Nams) in Human-Relevant Risk Assessment

Nicole Kleinstreuer[1], John Wambaugh[2], Joshua Harrill[2], Predrag Kukic[3]
[1]NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), NIEHS, Raleigh-Durham-Chapel Hill Area, USA. [2]US Environmental Protection Agency, Chapel Hill, USA. [3]Unilever, Bedford, United Kingdom

Abstract

New Approach Methodologies (NAMs) promise to offer a unique opportunity to enable human-relevant safety decisions to be made without the need for animal testing. The application of NAMs in risk assessment has gained much traction recently, and concrete examples of how to analyze, integrate and interpret NAMs to inform a human-relevant safety decision are beginning to appear in the literature, helping to increase the accessibility of the overall approach. However, as with any novel technology, the use, and data interpretation of NAMs might appear unfamiliar and complicated to non-specialists or novel users, limiting the extent to which industry and regulatory risk assessors can apply them with confidence. The aim of this continuing education course is to bring to life the use and application of NAMs in safety decision-making. The course is aimed to be accessible to the general ASCCT audience, and therefore no prior knowledge is required. The first session will focus on the current regulatory requirements for using NAMs and ongoing activities related to regulatory acceptance. The second and third session will give an overview of a range of NAMs that have already found their application in the exposure assessment, such as physiologically-based kinetic modelling, and in the bioactivity characterization, such as high throughput transcriptomics, high throughput phenotypic profiling, and safety pharmacology assays. In the final session, an example of an industry application of NAMs in safety decision making with regards to systemic toxicity in adults of benzophenone-4 as an ingredient in a sunscreen product will be illustrated.

First Presentation

Building Confidence in New Approach Methodologies (NAMs)
Nicole Kleinstreuer

A transformation in toxicological testing and regulatory decision making has been underway for several decades, embodied by a shift from an exclusive reliance on *in vivo* animal models to development and implementation of NAMs that may provide more rapid, efficient, and human-relevant information across a broad range of chemicals and endpoints. Evolving considerations around validation of NAMs have led to an increased emphasis on more flexible, fit-for-purpose approaches that consider regulatory needs and are tailored for particular contexts of use. This presentation will discuss current projects and future directions in building confidence in NAMs for safety decision-making, and how such efforts rely upon coordination across international partners and diverse stakeholders. Participants will learn about the regulatory perspective on validation and qualification of NAMs and be provided with examples of applying each essential element of a scientific confidence framework that has been agreed upon by multiple US federal agencies.

Second Presentation

Use of New Approach Methodologies (NAMs) in Human Exposure Modeling
John Wambaugh

NAMs can estimate points of departure (PODs) *in vitro* using bioactivity as a surrogate for in *vivo* hazard data. NAMs for exposure provide the context needed to understand *in vitro* PODs in terms of public health risk. *In vitro-in vivo* extrapolation (IVIVE) of PODs requires chemical-specific information on *in vitro* distribution and *in vivo* toxicokinetics (that is, absorption, distribution, metabolism, and excretion). Since most chemicals lack data, we use high throughput toxicokinetics (HTTK). HTTK is the combination of *in vitro* measurement of key determinants of toxicokinetics with generic toxicokinetic mathematical models. NAM-based PODs can then be compared with high throughput estimates of chemical intake to develop tentative bioactivity:exposure ratios. Participants will become familiar with key human exposure modeling approaches and the Systematic Empirical Evaluation of Models high throughput framework that uses Bayesian methods to incorporate multiple exposure models into consensus predictions.

Third Presentation

Use of New Approach Methodologies (NAMs) in Bioactivity Characterization
Joshua Harrill

This presentation will provide an overview of several types of *in vitro* NAMs that are being used for chemical bioactivity screening and *in vitro* hazard evaluation. Technologies discussed will include targeted *in vitro* NAMs such as a safety pharmacology and cell stress assay panels, targeted phenotypic assays as well as non-targeted high-throughput transcriptomic and phenotypic profiling assays. Examples will be provided explaining how information from these NAMs could be incorporated into fit-for-purpose risk assessment applications such as molecular point-of-departure determination and chemical grouping. Participants will also gain a more thorough understanding of how information from multiple *in vitro* screening NAMs can be integrated to inform chemical risk assessment.

Fourth Presentation

An Example of Application of New Approach Methodologies (NAMs) to Evaluate Systemic Safety for Consumers using Benzophenone-4 as a UV-filter in a Sunscreen Product
Predrag Kukic

In the final session, participants will learn a way to integrate the presented NAMs using a real case industry application to inform a human-relevant safety decision, judging uncertainty, weight-of-evidence, and regulatory context considerations. In particular, we will illustrate how *ab initio* safety evaluation could be done for a single cosmetic ingredient. NAM data for Benzophenone-4 used at 5% in a sunscreen body lotion product have been generated and the results will be presented with the purpose of deriving i) a predicted consumer systemic exposure concentration (SEC) of Benzophenone-4, to compare with ii) point(s) of departure using human-relevant NAMs (POD$_{NAM}$) which provide information on bioactivity of Benzophenone-4. A Bioactivity:Exposure Ratio (BER) was calculated as POD$_{NAM}$/SEC, and a decision to whether the BERs provide an adequate basis for safety assurance in the context of the ingredient use scenario will be explained.

CE4: Use of NAMs to Predict EC3 Values for Application in Skin Sensitization Risk Assessment

Argel Islas-Robles[1], John Yin[2], Andrew J Keebaugh[3]
[1]Institute for In vitro Sciences Inc., Gaithersburg, USA. [2]OPI, Wella Company, Calabasas, USA. [3]UES, Inc. and Air Force Research Laboratory, Wright Patterson Air Force Base, USA

Abstract

A skin sensitizer is a chemical which leads to an inflammatory reaction (allergic contact dermatitis) through repeated exposure. Identification of sensitizers has become of prominent importance due to the increased understanding of the many chemicals that humans interact with on a daily basis. Therefore, characterizing the hazard and health risk of sensitizers is needed. Historically, animal models have been used to characterize sensitizers, such as the mouse local lymph node assay (LLNA). Based on to the need to use human-relevant models and due to ethical concerns, new approach methodologies (NAMs) have been developed to assess this endpoint. These include a combination of *in silico*, *in chemico*, and *in vitro* methods which are used in a tiered approach. Various NAMs have been validated, adopted by the OECD, and recognized by regulatory agencies worldwide. More recently, NAM-based data have been leveraged to derive a point of departure for next-generation risk assessment. One promising approach is the prediction of LLNA EC3 values (dose leading to stimulation index of 3.0; considered threshold for positive sensitization) using linear regression models based on data from OECD-validated methods (k-DPRA, Keratinosens and/or h-CLAT). For this continuing education course, participants will learn the key steps to establishing predicted EC3 from NAM-based data with a focus on special cases, and will understand their application in risk assessment via presentations of real life examples and a round table discussion which overall will contribute to expand the applicability of the established method beyond the cosmetic and consumer product ingredients.

First Presentation

Overview of NAMs & Regression Models to Derive Predicted EC3
Argel Islas-Robles

Historically, animal models have been considered the gold standard to assess the human risk to skin sensitizers. More recently, models that do not use animals, or new approach methodologies (NAMs), have been developed and their use is gaining acceptance for hazard identification. In 2022 Natsch & Gerberick developed a method based on NAMs' data and linear regression models to calculate a point of departure for application in risk assessment in the form of predicted LLNA EC3 values (pEC3). This method allows the establishment of continuous potency data with results obtained from the OECD-validated methods: kinetic direct peptide reactivity assay (k-DPRA), the KeratinoSens™ (KS) assay, and/or the human cell line activation test (h-CLAT). This presentation will include an overview of the NAMs, OECD defined approaches for skin sensitization, the regression models, and how to use these for the generation of pEC3 with a focus on special cases.

Second Presentation

Prediction of EC3 Values for Nail and Hair Cosmetic Ingredients Using In Vitro Test Data for Quantitative Risk Assessment
John Yin

Over the past two decades, there has been an increasing trend towards assessing cosmetic ingredients and finished products using *in vitro* data. Recently, several linear regression models have been developed that allow prediction of EC3 as from LLNA as point of departure. In this study, nail and hair care ingredients were tested in k-DPRA, Keratinosens assay, and h-CLAT and their EC3 values were predicted with the linear regression models. The predicted EC3 values were then compared with the historical LLNA EC3 data. There was a strong positive linear relationship between the predicted EC3 values and historical LLNA EC3 data. The results of this study support the use of the linear regression models and the *in vitro* data to predict EC3 values for quantitative risk assessment. The results also demonstrate the flexibility of using a cell-based assay in place of k-DPRA when data of the latter are not available.

Third Presentation Title

Development of Preliminary Candidate Surface Guidelines for Air Force-relevant Dermal Sensitizers using New Approach Methodologies
Andrew Keebaugh

This study aimed to better characterize the potential link between allergic contact dermatitis (ACD) and chemical exposures during AF operations by 1) evaluating the sensitization potential of AF-relevant chemicals using new approach methodologies (NAMs), and 2) developing preliminary candidate surface guidelines (PCSGs, i.e., guidelines for maximum surface concentrations to prevent induction of sensitization) using NAM data. k-DPRA and KeratinoSens™ assays were used to predict LLNA effective concentration values (EC3) via the method of Natsch & Gerberick (2022). The assay results, in silico models, and available human and animal data were also leveraged into an integrated approach to predict sensitizer status for each chemical based on weight of evidence. PCSGs were derived by adjusting the predicted EC3 values to occupationally-relevant surface concentrations for chemicals predicted to be sensitizers. PCSGs can be compared to measured surface concentrations of potential sensitizers to better understand the risk of Airmen developing ACD from occupational exposures.

Round Table Discussion

Attendees will have the opportunity to interact and discuss the presented topics with the presenters, including:

- how the information from the NAMs was used in each training example;
- use and value of pEC3 to generate decisions by each presenter's organization;
- and lessons learned from these applications, and how can these lessons best be applied in the development, evaluation and use of NAMs.

Additionally, Test cases with a focus on special situations will be shared in order to promote the discussion of pEC3 generation and use in Risk Assessment.

Oral Presentations

OR1

Human Liver-Chips Have the Potential to Improve the Patient Safety of Pharmaceuticals: A Predictive and Economic Analysis

Lorna Ewart
Emulate, Boston, USA

Abstract

Assuring the safety of a new medicine prior to human exposure is essential. Despite best efforts, safety concerns still drive significant drug attrition during clinical trial or removal from the marketplace, with the liver as a leading target organ for toxicity. Indeed, since January 2022, there have been at least 7 clinical trials or on-market drugs that were impacted by unexpected reports of drug-induced liver injury (DILI). This highlights the need for continued development of human-relevant predictive tools using 21st century approaches, such as organ-on-a-chip. My presentation will introduce why we should challenge the current status quo of preclinical safety testing for hepatotoxicity, how Liver-Chip has been characterized as a predictive tool with 87% sensitivity and 100% specificity and importantly how quantitative data from this model can be used in preclinical decision making at the point of progressing a candidate drug into Phase I clinical trial. I will conclude with a few remarks on how usage of this tool has economic benefits that in turn can increase R&D productivity.

OR2

Hepatic Spheroids for High-Throughput Predictive Hepatotoxicity Assessment During Pharmaceutical Drug Development

Madhu Nag, Anna Borgstroem, Bruno Filippi, Sue Grepper
InSphero AG, Schleiren, Switzerland

Abstract

Despite extensive pre-clinical and clinical testing, many drugs are withdrawn from the market due to safety liabilities. Toxicity testing of drug candidates in animal models is endorsed by regulatory authorities, but they do not always predict human outcome. The recent development of micro-physiological systems raises hopes that their incorporation in the safety assessment process will help improve hepatotoxicity evaluation. Hepatic spheroids are co-cultures of primary human hepatocytes and non-parenchymal cells that recapitulate important features of a native liver. Moreover, they predict hepatotoxicity more accurately than 2D primary hepatocyte cultures. To further evaluate the relevance of the hepatic spheroids for hepatotoxicity assessment, the cytotoxicity of 171 FDA-approved small molecule drugs in hepatic spheroids was compared with their in vivo hepatotoxicity and total plasma cMax. The incorporating of human exposure data significantly increased the sensitivity and specificity of the hepatic spheroids, with 84.2% of "Most-DILI-Concern" drugs accurately predicted as hepatotoxic and

83.3% of "No-DILI-Concern" drugs are correctly predicted as non-hepatotoxic. The correlation between the in vitro cytotoxicity and the in vivo hepatotoxicity of the tested drugs shows the relevance of hepatic spheroids for liver safety assessment. Their physiological features combined with their scalability, cost-efficiency and standardized production makes hepatic spheroids a reliable and industry-compatible liver model for safety assessment.

OR3

Practical Application of New Approach Methods in Developmental and Reproductive Toxicity Testing

Predrag Kukic[1], Paul Carmichael[1], Matthew Dent[1], Jade Houghton[1], Amer Jamalpoor[2], Luke Flatt[2], Hequn Li[1], Alistair Middleton[1], Gopal Pawar[1], Claire Peart[1], Katarzyna Przybylak[1], Magdalena Sawicka[1], Sandrine Spriggs[1], Ramya Rajagopal[1], Katy Wilson[1], Kathryn Wolton[1], Iris Muller[1]
[1]Unilever, Bedford, United Kingdom. [2]Toxys, Leiden, Netherlands

Abstract

New Approach Methodologies (NAMs) offer a unique opportunity to enable human-relevant safety decisions to be made without the need for animal testing. Encouraged by the successful application of NAMs to an exposure-led safety assessment for systemic toxicity, an integrated framework to include additional NAMs covering specific DART-related biology was developed. The framework comprised DART-specific in silico predictions, in vitro physiologically based kinetic (PBK) modelling, a panel of biomarkers related to general xenobiotic cell stress pathways, high-throughput transcriptomics, a panel of secondary pharmacology assays, as well as ReproTracker® and devTOX quickPredict™ assays to address developmental toxicity. To determine if the proposed DART framework was sufficiently protective for consumer safety assessments, first its biological coverage was evaluated. The comparison between reported cellular processes, signaling pathways and genes involved in key stages known to be important in human reproduction/embryo-foetal development to the read-outs from our DART framework showed >80% coverage based on gene biomarker numbers. In addition, the DART framework was evaluated using data generated on 40 benchmark substances. The points of departure from concentration-response curves in the in vitro assays were compared to exposure estimates to calculate bioactivity-exposure ratios (BERs) for each substance. To evaluate the approach, these BERs together with in silico predictions were compared to literature reports of the DART risk for each substance. The distribution of BER values will be presented together with areas identified where integration of additional NAMs could be beneficial to further build confidence in human-relevant safety decision making.

OR4

Leveraging the Success of the DASS to Establish IATAs for Respiratory Sensitization

Jessica Ponder[1], Emily Reinke[2], Kristie Sullivan[3], Eryn Slankster-Schmeirer[1], Rob Vandebriel[4], Elizabeth Baker[1]

[1]Physicians Committee for Responsible Medicine, Washington, USA. [2]Inotiv, Morrisville, USA. [3]Institute for In Vitro Sciences, Gaithersburg, USA. [4]National Institute of Public Health and Environment, Bilthoven, Netherlands

Abstract

There is a regulatory need for internationally harmonized approaches to identify chemical respiratory allergens to protect worker and consumer health. This presents a unique opportunity to apply nonanimal methods and human biological understanding ab initio to testing approaches that address sensitization of the respiratory tract for accurate hazard and risk assessment. Towards this end, an Adverse Outcome Pathway has been assembled to support the development and evaluation of methods. This AOP follows a similar path to dermal sensitization, from protein binding to immune activation. However, these pathways diverge at early key events, leading to IgE-mediated bronchial hypersensitivity for respiratory sensitizers rather than T cell-mediated contact dermatitis. Thus, human-relevant testing approaches will differ from those described for skin sensitization. Several efforts have emerged to tackle this ongoing issue. To harmonize these efforts and involve regulatory agencies early in the development of approaches for respiratory sensitizers, several organizations and agencies have initiated a review paper within the OECD. This review will outline the regulatory needs, state of the science, and technical expectations of test methods in development, laying the groundwork for regulatory instruments, such as Integrated Approaches for Testing and Assessment (IATA) or test guidelines to be drafted in the future. These efforts continue the path trailblazed by the establishment of the OECD Guideline 497, Defined Approaches for Skin Sensitization (DASS), while breaking new ground as a toxicological endpoint without an existing in vivo benchmark. ENR's work was funded with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.

OR5

An FDA/CDER Perspective on Nonclinical Testing Strategies Including New Approach Methodologies

Ilona Bebenek
Food and Drug Administration, College Park, USA

Abstract

Recent advances in science have led to the emergence of numerous new approach methodologies (NAMs) for nonclinical testing that are currently being used in various aspects of drug development. Traditional nonclinical testing methods can predict most clinical outcomes, although improvements in these methods that can increase predictivity of clinical outcomes are encouraged and needed. The goal of this presentation is to discuss NAMs, and considerations in their use to support the overall safety assessment of pharmaceuticals, in the context of regulatory development.

OR6

Implementing the ICCVAM Roadmap: Establishing Confidence

John Gordon
Consumer Product Safety Commission, Bethesda, USA

OR7

NASEM Report: Building Confidence in New Evidence Streams for Human Health Risk Assessment

Marie C Fortin
Jazz Pharmaceuticals, Philadelphia, USA. Rutgers University, New Brunswick, USA. NASEM Committee Member, Washington, USA

Abstract

Historically, the most influential toxicological data have come from laboratory mammalian studies, which still form the foundation of most human health assessments. Over the past decade it has become increasingly clear that relying primarily on laboratory mammalian studies limits the ability to assess the human health hazards and potential risk of the tens of thousands of chemicals to which people may be exposed. New approach methods (NAMs) in toxicology offer opportunities to surmount at least some of these limitations. For instance, NAMs could potentially inform timely decision-making when no other data are available. They may also allow to characterize subtle health perturbations or better encompass biological diversity. Although the promise and need for NAMs is clear, many barriers to their use remain and few concrete examples exist today of NAM data applications in hazard or risk assessment decisions. This NASEM report aimed to bridge this notable gap between the potential of NAMs and their practical application in human health risk assessment.

The report identified five common components of evaluating scientific confidence of NAMs: (1) intended purpose and context of use, (2) internal validity, (3) external validity, (4) experimental and biological variability, and (5) transparency. A key aspect of the committee's recommendations to build a bridge from NAMs to application in human health risk assessment involves use of a population, exposure, comparator, and outcomes (PECO) statement. A cornerstone of systematic review approaches, the PECO statement clarifies the question being addressed and promotes transparency. For instance, laboratory mammalian toxicity tests are generally intended as surrogates for a corresponding "target human" PECO for the same biological tissue or system. The report recommends that EPA address this gap by defining a "target human" PECO for each NAM, thereby providing information as to how it would inform human health hazard identification or dose-response.

Overall, the report envisions that the development and utilization of a framework that integrates these new evidence streams will enable the use of NAMs to address the lack of data for a vast number of chemicals, better cover susceptible and vulnerable populations, and thus ultimately improve protection of public health.

OR8

EPA Draft Report on Statutory and Regulatory Requirements for Vertebrate Animal Testing and Flexibility for Implementing New Approach Methods (NAMs)

Anna B Lowit
US EPA, Washington, USA

OR9

Software and Database Enhancements to ToxCast for Integration and Use of in Vitro Bioactivity Screening Data

Madison Feshuk[1], Jason Brown[1], Sarah Davidson-Fritz[2], Kelly Carstens[1], Carter Thunes[3,1], Ashley Ko[3,1], Richard Judson[1], Katie Paul Friedman[1]
[1]Center for Computational Toxicology and Exposure, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC, USA. [2]Center for Computational Toxicology and Exposure, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH, USA. [3]Oak Ridge Associated Universities National Student Services Contract, Oak Ridge, TN, USA

Abstract

The US Environmental Protection Agency Toxicity Forecaster (ToxCast) program makes in vitro medium- and high-throughput screening assay data publicly available for chemical prioritization and hazard characterization. The ToxCast data pipeline (tcpl) is an open-source R package that stores, manages, curve-fits, and visualizes ToxCast data while populating the linked MySQL database, invitrodb. Evolving data needs for next generation risk assessment necessitated software and database updates for analogous data processing and management across multi-concentration screening efforts. Implementation of a single curve-fitting approach as encoded by the R package tcplfit2 motivated changes that promote comparability across multiple tiers of bioactivity data within the CompTox Blueprint, including targeted ToxCast assays (Tiers 2-3) and broad profiling assays in Tier 1. Major updates to tcpl and invitrodb include modified annotation structure, additional models, bidirectional curve-fitting, continuous hit calling. Fit categories and cautionary flags were also updated to reflect new curve-fitting behaviors with tcplfit2. These updates resulted in minimal overall changes in activity hit calls and potency estimates in invitrodb version 4.0 compared to the previous invitrodb version 3.5, with most changes seen in borderline responses. ToxCast provides a standard for consistent and reproducible data pipelining for diverse, targeted in vitro assay data with readily available documentation and a unified open-source software approach. These updates further enable use of these data in a myriad of toxicology applications within an integrated data landscape. This abstract does not necessarily reflect U.S. EPA policy.

OR10

Expanding PBPK Modeling to Predict Chemical Distribution in Brain and Adipose Tissues

Aswani Unnikrishnan[1], Xiaoqing Chang[1], Victoria Hull[1], Getachew Ebuy Tedla[1], Helena Hogberg-Durdock[2], James Sluka[3], John Wambaugh[4], David Allen[1], Nicole Kleinstreuer[2]
[1]Inotiv, RTP, USA. [2]NIH/NIEHS/DTT/PTB/NICEATM, RTP, USA. [3]Indiana University, Bloomington, USA. [4]U.S.EPA/ORD/CCTE, RTP, USA

Abstract

To facilitate decision making in drug discovery and risk assessment, physiologically based pharmacokinetic (PBPK) modeling is used for high-throughput applications. Most existing open-source PBPK models predict chemical concentrations in major body compartments such as liver, kidney, and gut. However, organ-specific toxicological effects require specialized models. For example, models are needed to facilitate neurotoxicity evaluations by predicting chemical distribution to the brain, which has complex structural features and the potential for significant adverse effects. Incorporating the blood-brain barrier in a PBPK model and evaluating whether a chemical can cross this barrier is an important first step in assessing the potential neurotoxicity of the chemical. Another limitation of existing open-source PBPK models is that they often do not include an adipose tissue compartment. Adipose tissue plays a critical role in toxicokinetics by acting as a storage compartment for lipophilic chemicals and a source of continuous internal exposure as the chemical is released. In this study, we added brain and adipose tissue compartments to the existing generic PBPK model from the httk R package (v2.2.2), developed by the U.S. Environmental Protection Agency, to better estimate chemical concentrations in these two tissues. The Open (Quantitative) Structure-activity/property Relationship App's (OPERA) lipophilicity predictions provided estimates on the propensity for accumulation of chemicals in adipose tissue. This presentation describes the creation of this new model and its future implementations in the field of toxico- and pharmacokinetics. This project was funded by the NIH NIEHS under Contract No. HHSN273201500010C and by EPA under Contract No. RD840027.

OR11

Deriving Benchmark Dose from the Deep Learning Prediction Scores of High-Throughput Toxicology Images

Arpit Tandon[1], Brian E Howard[1], Deepak Mav[1], Michele Balik-Meisner[1], Sreenivasa Ramaiahgari[2], Stephen Ferguson[2], Ruchir R Shah[1], B. Alex Merrick[2]
[1]Sciome LLC, Research Triangle Park, USA. [2]National Institute of Environmental Health Sciences (NIEHS), National Toxicology Program (NTP), Research Triangle Park, USA

Abstract

We have developed a deep learning approach to classify digital assay images according to the health of the cells found in each well. Expert annotators classified 2,160 high-resolution assay images from differentiated 2D cultures of HepaRG cells, treated with 24 chemicals, 10 doses and 9 replicates each.

Images were categorized into 2 classes: healthy and altered. We then used leave-one-out cross validation to train a convolutional neural network to perform binary classification of unlabeled images. Models were trained iteratively on the images corresponding to 16 chemicals while validation was performed on the images for the remaining 8 chemicals. Next, we constructed a model that is able to classify assay images into healthy and altered classes with >98% accuracy. The classification scores are then used to calculate BMD values after transforming classification score into a z-score using the Probit function. These transformed scores were analyzed using BMDExpress2.3 to perform dose response predictions. We observed >90% Pearson's correlation between the image-derived BMD values and those calculated using the corresponding transcriptomic dataset. We performed pathway enrichment on genes showing high correlations between image and transcriptomic BMDs. We observed apoptosis, cell death, necrosis, etc. as the top enriched pathways. Together, these results suggest that BMD scores derived from high-throughput imaging might be used as an alternative faster and less resource intensive method to calculate benchmark doses. This approach might be useful, for example, during the experimental design phase in order to suggest appropriate dosage ranges to use when developing more comprehensive transcriptomic experiments.

OR12

The SARA-ICE Model for Predicting Skin Sensitizer Potency

Gavin Maxwell[1], Georgia Reynolds[1], Joe Reynolds[1], Nicola Gilmour[1], Judy Strickland[2], Emily N Reinke[2], Dori Germolec[3], Jim Truax[2], Dave G Allen[2], Nicole Kleinstreuer[3]
[1]SEAC, Unilever, Bedford, United Kingdom. [2]Inotiv, Durham, USA. [3]NIH/NIEHS/DTT/PTB/NICEATM, Durham, USA

Abstract

The Skin Allergy Risk Assessment-Integrated Chemical Environment (SARA-ICE) model is a probabilistic defined approach (DA) that provides a weight-of-evidence point of departure (PoD) and GHS potency prediction for use in skin sensitization assessments. SARA-ICE is constructed within the Bayesian statistical framework using data sourced from the Integrated Chemical Environment (https://ice.ntp.niehs.nih.gov/), Unilever SARA publications, and Cosmetics Europe. SARA-ICE predicts a human relevant PoD: the ED01, the dose with a 1% chance of inducing sensitization in a human predictive patch test (HPPT). The PoD can be calculated using data from HPPTs, local lymph node assays (LLNA), and new approach methodologies (NAMs). For a chemical of interest, the model returns the probability of each GHS classification conditional on the distribution of the ED01. We used the OECD DA for Skin Sensitisation (TG 497) reference data set to evaluate SARA-ICE for GHS classification accuracy. Using a probability of 0.8 as the binary classification criterion, balanced accuracy was 95% for conclusive calls relative to human classifications, but 32% of chemicals had inconclusive calls. A case study on isothiazolinones demonstrated that SARA-ICE performed well, correctly identifying these broad-spectrum preservatives as sensitizers. SARA-ICE will be made freely available online, enabling users worldwide to easily predict human skin sensitization potency without animal testing and supporting probabilistic risk assessment applications. This project was partially funded by NIEHS under Contract No. HHSN273201500010C.

OR14

ChemMaps.com v2.0 - Exploring the Environmental Chemical Universe

Alexandre Borrel[1], Aswani Unnikrishnan[1], Dave G. Allen[1], Nicole Kleinstreuer[2]
[1]Inotiv, RTP, USA. [2]NIH/NIEHS/DTT/PTB/NICEATM, RTP, USA

Abstract

Access to visualization tools to navigate chemical space has become more important due to the increasing size and diversity of publicly accessible databases and associated compendiums of high-throughput screening (HTS) and other descriptor and effects data. Construction of such tools relies on complex projection techniques using molecular descriptors. However, application of these techniques requires advanced programming skills that are beyond the capabilities of many stakeholders. Inspired by the popular Google Maps application, we developed the ChemMaps.com webserver (https://sandbox.ntp.niehs.nih.gov/chemmaps/) to easily navigate chemical space. The first version of ChemMaps.com enabled users to browse and visualize a space of 2,000 FDA-approved drugs and over 6,000 drug candidates from the DrugBank database (https://www.drugbank.ca/). The chemical space of ChemMaps.com v2.0, released in 2022, adds data on approximately one million environmental chemicals from the EPA Distributed Structure-Searchable Toxicity (DSSTox) inventory. ChemMaps.com v2.0 incorporates mapping to HTS assay data from the U.S. federal Tox21 research collaboration, which includes results from approximately 2,000 assays tested on up to 10,000 chemicals. ChemMaps.com v2.0 users can visualize chemical activity both by assay and target directly on the map and compare chemical spaces occupied by active and inactive chemicals. ChemMaps.com v2.0 also has new navigation options, including an on-the-fly distance measurement between two chemicals selected on the 3D map, a map screenshot button, and customizable color mapping based on chemical properties. Project was funded by NIEHS under Contract No. HHSN273201500010C.

OR15

Establishing a Fully Automated Image-Based Assay for Detecting Tubulin Stabilizing and Destabilizing Effect With a Supervised Machine Learning Approach

Wei Chen, Yang Li, Audrey Lehman, Kristina Johnson, Heather Pence, Lori Breitweiser, Adrew Bowling, Eric Sherer, Jessica LaRocca
Corteva Agriscience, Indianapolis, USA

Abstract

Microtubules are a major part of the cytoskeletal system of eukaryotic cells and play a critical role in a wide variety of cellular processes. Xenobiotics that alter microtubule structures often have deleterious effects on cells, and in consequence can result in genotoxicity, testis toxicity, and developmental toxicities in humans and other animals. Image-based assays have been reported on tubulin effect for risk assessment and cancer treatment. In this study, we optimized an immunocytochemistry-based method in combination with high content imaging to visualize the microtubule filaments and developed an

automated process to evaluate the microtubule disruption on a metabolic competent human liver cell line, HepaRG. A total of 33 microtubules stabilizing, 35 destabilizing, and 100 neutral compounds with distinguishable phenotypes were carefully chosen as the training library. Four-hundred and ninety-four cell morphology and tubulin-related features were extracted using Harmony® image analysis software from individual cells. The initial principal component (PC) accounted for 81.2% of the overall variance while the second and third PCs accounted for 8.1% and 5.8% of the variance, respectively. By utilizing the top 3 PCs, all three machine learning algorithms tested - Extreme Gradient Boosting (XGBoost), Random Forest (RF) and Support Vector Machine (SVM) achieved impressive accuracy rates of over 97% and precisions of 95% in 5-fold cross-validation. This approach has been incorporated for early detection of genotoxicities to enhance the risk assessment and aid in the design of sustainable crop protection products.

OR16

Assessing Sunscreen Product Toxicity on Coral Fragments: A New Approach Methodology for Enhancing Reliability and Addressing Environmental Concerns

Dâmaris Cristine Marios Ferreira Pinto[1], Natalia De Albuquerque Vita Abreu[1], Gabriela De Oliveira Prado Correa[1], Fernanda Cristina Massaro[2], Denise Tieme Okumura[2], Desiree Cigaran Schuck[1], Andrezza Di Pietro Micali Canavez[1]
[1]Grupo Boticário, São José dos Pinhais, Brazil. [2]Biotox Análises Ambientais, São Carlos, Brazil

Abstract

Concerns have arisen regarding sunscreen ingredients and their potential impact on coral reefs. The scientific community remains uncertain about the extent of these risks, while regulatory decisions lack robust scientific evidence. Consequently, environmentally-conscious consumers seek "reef-safe" cosmetic products, yet the absence of standardized guidelines for defining reef-safe sunscreens creates ambiguity for both consumers and manufacturers. To address these challenges, this study proposes a novel New Approach Methodology (NAM) for assessing sunscreen product toxicity on coral fragments and supporting regulatory agencies in establishing clear criteria for reef-safe evaluation. The NAM adopts a holistic perspective, evaluating the entire product composition rather than solely focusing on excluding controversial ingredients. By adopting this comprehensive approach, the NAM aims to enhance the reliability of toxicity assessments concerning coral ecosystems. Seven sunscreens with varying Sun Protection Factors (SPF) were evaluated. The acute toxicity in coral fragments (*Seriatopora Hystrix*) was assessed by exposing them to higher concentrations than those found in the natural environment. Results were evaluated based on coral fragment retraction and bleaching levels, with bleaching assessment performed by measuring the RGB (red, green, and blue) variance of the samples. While two samples showed toxic effects at different concentrations, five samples exhibited no adverse effects on the coral fragments, even at the highest tested concentration. The proposed NAM could play a crucial role in addressing the concerns of environmentally-conscious consumers, based on robust evidence, as we strive for a more sustainable future.

OR13

Application of Bioinformatics for Species Extrapolation in Regulatory Decision-making as an International Collaboration

Carlie LaLone
US EPA, Duluth, USA

Abstract

The global regulatory landscape surrounding chemical safety is shifting away from animal testing and moving toward greater reliance on New Approach Methodologies (NAMs) including a rapid growth in molecular data and computational methods to achieve human and environmental protection goals. This change presents both new hurdles and opportunities for advancing NAMs focused on a non-animal testing agenda and accelerating them into mainstream regulatory decision-making. One challenge in this regard is understanding how existing data on effects observed in few species, typically model test species, translate to the diversity of life and so can be used as surrogate information across a broader biological domain, reducing the need for additional data generation. The adverse outcome pathway (AOP) conceptual framework and descriptions of the taxonomic domain of applicability for key events (KEs) and key event relationships (KERs) through the biological levels of organization provide an opportunity to integrate evidence of structural and functional conservation, which can be gleaned from computational methods. To capitalize on advances in OMICs technologies and take advantage of existing toxicity data that can be generated without the use of whole animal models (e.g., high throughput screening and transcriptomics data, in vitro, and fish embryo testing), approaches in bioinformatics are starting to demonstrate application for cross species extrapolations. Bioinformatics applies computational techniques to understand and organize information associated with molecules, including sequence and structural alignments, phylogeny evaluations, prediction of protein structures and function, gene annotation, and gene expression analyses, as examples, to understand biology and biological pathways. Advances in bioinformatics have begun to inform chemical safety evaluations, specifically informing species extrapolation. Tools and methods for species extrapolation that are built with bioinformatic approaches have been peer reviewed, published, and publicly accessible to enable their use in research and regulatory decision-making. To advance cross species extrapolation with an objective to inform a 21st century regulatory non-animal testing agenda for assessing human and ecological health, a global, cross-sector consortium, The International Consortium to Advance Cross Species Extrapolation in Regulation (ICACSER; https://www.setac.org/page/scixspecies), has been created with the researchers, regulators, and advocates working toward integration of approaches in bioinformatics. Due to the need for toxicity knowledge for a larger diversity of species it is timely to bring together a global consortium to focus efforts. While recognizing the importance of toxicokinetics in understanding and applying cross species extrapolation approaches, the primary initial focus of the consortium is on capitalizing on the rapidly expanding opportunities in bioinformatics methods for advancing regulatory decision-making. If successful, this will allow risk assessors to make better use of existing toxicological information and more easily consider the impact of chemicals on a variety of species.

OR17

Using New Approach Methods for Evaluation of Complex Mixtures: Addressing the Safety of Consumer Products and Botanical Extracts

Jorge M. Naciff
Procter and Gamble, Cincinnati, USA

Abstract

Human exposure to complex chemical mixtures occurs on a daily basis, which it happens from the use of personal care products to botanical extracts used as dietary herbal supplements or even medications. The use of these products is based on the premise that they are safe to use and do not pose any significant health risk. In the case of personal care products, the mixtures are very well characterized and their safety assessment is relatively straightforward. However, in some instances, there is a need to elucidate the potential interaction of some of the components of the mixture, acting by similar modes of action, or to gather some data to fill data gaps for individual chemicals. In this case, the use of new approach methodologies (NAMs), which include in vitro assay-based testing of chemicals and in silico models and tools, can be useful. The safety assessment of botanical extracts is significantly more challenging since their composition is more complex and in multiple cases includes hundreds of chemicals with various modes of action and limited or nonexistent safety data. In this case, the use of in silico and in vitro approaches to gather data usable in the assessment of this complex mixtures is significantly valuable. Case studies describing the application of NAMs to complex chemical mixtures will be presented, with particular focus on the use of analytical chemistry to identify major components, coupled with the use of in vitro bioassays to determine the biological activity of the mixture, mostly transcriptional profiling and targeted high-throughput receptor binding and enzyme activity screening, in combination with exposure estimates to derive screening-level metrics for quantitative assessment of risk based on potency comparisons.

OR18

How Does Stress Modify Chemical Toxicity? A High-Throughput In Vitro Screening Approach to Advance New Approach Method (NAM) Testing of Differences in Susceptibility

Laura Word[1], Clinton Willis[1], Stephanie Padilla[1], Chelsea Weitekamp[1], Kelly Carstens[1], Logan Everett[1], Bridget Knapp[1,2], Richard Judson[1], Joshua Harrill[1]
[1]U.S. Environmental Protection Agency, RTP, NC, USA. [2]Oak Ridge Institute for Science and Education (ORISE), Oak Ridge, TN, USA

Abstract

People living in communities that are subjected to a disproportionate burden of environmental hazard and/or who experience a significantly reduced quality of life relative to surrounding or comparative communities (Environmental Justice Communities of Concern) can experience higher levels of chronic

stress that could change their response to environmental chemical exposures. To address this potential interaction, we are developing an in vitro New Approach Methodology (NAM) to evaluate chemical effects at different levels of the stress hormone, cortisol. We are phenotypically profiling chemical effects in human osteosarcoma cells that express fluorescent protein markers for the nucleus and cytoskeleton (U-2_OS_FP). The U-2_OS_FP cells express glucocorticoid receptor (the target of cortisol) and we transitioned the cells to a cortisol-defined and serum-free media for the experiments. The cells are being co-exposed to a mixture of cortisol, at baseline and elevated concentrations, plus toxicants in a time- and concentration-dependent screen to evaluate cellular phenotypic changes. We are screening 147 chemicals. These chemicals are relevant to environmental justice (EJ, n=73), impact the glucocorticoid receptor (n=22) or overlapping gene pathways (n=25), are carcinogenic (n=20), endocrine disruptors (n=15), and/or induce cell stress responses (n=13). These studies begin to address concerns in EJ Communities by creating a novel NAM model for how stress may impact adverse outcomes from chemical exposure. *The views are the authors' and do not necessarily represent the views of the U.S.EPA.*

OR19

Development of a Novel Milli-fluidic Liver Tissue Chip (LTC) Platform for Mechanistic Preclinical Studies

Shiny Rajan, Murat Cirit
Javelin Biotech, Woburn, USA

Abstract

The estimation and optimization of drug properties to develop efficacious and safe therapies for humans is a critical step in preclinical discovery. After the FDA Modernization Act 2.0, the need for in vitro alternative technologies has been drastically increased. Human tissue chips, aka organs on chips, provide a platform to develop new alternative methodologies (NAMs). However, most common tissue chip technologies are microfluidic-based tissue chips, which have limited utility to develop NAMs for mechanistic safety pharmacology studies.

We designed LTC (larger tissue size and media volume than micro-fluidics) with continuous recirculation for mechanistic pharmacology studies and multi-scale data generation (media- and tissue level). We evaluated primary human tissue model stability & function over 15 days (morphology, clinical biomarkers, gene expression and metabolic activity) and compared these results with clinical values. Pharmacokinetics (PK) of over 15 drugs has been evaluated by parent drug depletion and metabolite formation. PK studies were performed as individual and drug cocktails (mixtures). These studies demonstrated high in vitro in vivo correlation (IVIVC), especially for low clearance compounds, and accurate clinical exposure predictions with physiologically-based pharmacokinetic (PBPK) modeling. Biotransformation studies confirmed clinically-observed metabolite formation. Dose escalation studies resulted elevated ALT levels and reduced albumin & urea production rates for DILI-positive compounds compared to compounds with less DILI concerns.

The preliminary data demonstrated the utility of this platform to advance mechanistic understanding how pharmaceutical drugs and environmental toxicants will perform with human physiology.

OR20

Medical Device Development Tools Program

Jessica Mavadia-Shukla
US Food and Drug Administration, College Park, USA

OR21

Evaluating Cytotoxicity of Medical Devices Developed for Bone Defects: Which Cell to Select?

Fahimeh sadat Tabatabaei
iFyber, Ithaca, USA

Abstract

In vitro cytotoxicity testing is an indispensable part of developing new biomaterials, including those designed for bone defects. The standard ISO 10993-5 recommends using L929 murine fibroblast-derived cell line for evaluating the cytotoxicity of medical devices. However, most researchers prefer "osteoblast-like cells" for this purpose.

This study aimed to analyze the diverse cells employed for evaluating the cytotoxicity of osteoinductive/osteoconductive biomaterials. Thirty-six articles that were published between 2013 and 2023 met the inclusion criteria. Various parameters of testing methods were discussed, including the cell used, culture conditions, mode of evaluation, and biological endpoints assessed in each study.

Various studies have utilized different types of primary cells or cell lines to assess the cytotoxicity of biomaterials intended for bone defects. A critical analysis of these studies highlights that in vitro cytotoxicity is significantly influenced by the cell type used. Consequently, to ensure a more comprehensive evaluation of the biological outcomes of these biomaterials, it is recommended to standardize the selection of cells, the medium employed, the cell density, and the most suitable method of interaction between the medical device and cells. Additionally, integrating new technologies, such as 3D tissue models, holds promise in replicating physiologically relevant conditions and providing a more accurate simulation of the clinical environment.

OR22

How to Qualify Nonanimal Methods Through Fda's Medical Device Development Tools Program

Andrew D. Nguyen[1], Jeffrey Brown[1], Gertrude-Emilia Costin[2], Shabnam Solati[3]
[1]PETA Science Consortium International e.V., Stuttgart, Germany. [2]Institute for In Vitro Sciences, Gaithersburg, USA. [3]MAT Biotech B.V., Abcoude, Netherlands

Abstract

FDA's Medical Device Development Tools (MDDT) program is a pathway that can be used to qualify nonanimal methods to evaluate medical devices and to obtain FDA feedback when seeking regulatory uses of a new or developing method. Once a nonanimal method is qualified, users can submit its data to the FDA without additional qualification or validation for all devices in the same context of use defined during qualification. We summarize two ongoing MDDT projects to advance nonanimal methods and replace the use of animals in compulsory biocompatibility assessments of (1) vaginal irritation and (2) pyrogen contamination, each with a narrow context of use. To replace the rabbit vaginal irritation test, preliminary data supports the use of a human reconstructed vaginal tissue model (EpiVaginal) to predict levels of vaginal irritation on water-based personal lubricants. More data is being collected to support this MDDT. To replace the rabbit pyrogen test, we evaluated the use of the Monocyte Activation Test (MAT) to detect pyrogen contamination of dental bone screws made of titanium or stainless steel. We also tested the MAT's ability to detect material-mediated pyrogens (MMPs) identified in ISO 10993-11:2017. We found that the MAT detects widely recognized pyrogens of concern from microbial origin and also detects some MMPs. This MAT MDDT was submitted to the FDA for review and the supporting data and justifications showcase the suitability of using the MAT for pyrogenicity assessments of medical devices.

Poster Presentations

P01

Facilitating Analysis of Implicit Uncertainties in QSAR prediction of Chemical Toxicity: A Case Study of Neurotoxicity

Jerry Achar, Gunilla Öberg
The University of British Columbia, Vancouver, Canada

Abstract

Characterizing and communicating uncertainties in quantitative structure-activity relationship (QSAR) predictions are important to improve confidence in such predictions, yet rarely done. This case study aims to test if verbally expressed uncertainty indicators (i.e. possibly, it is thought that) can be used to identify uncertainties and operationalize a framework designed to categorize uncertainties, thereby explicit-make implicit uncertainties in QSAR predictions. Using the neurotoxicity endpoint as our case, we selected fifteen peer-reviewed scientific publications on QSAR prediction for analysis. The texts were analyzed line-by-line to identify uncertainty indicators. By interpreting the context in which the indicators were used in the text, the indicators were then used to identify uncertainties. The identified uncertainties were categorized using the said uncertainty categorization framework, and the relative frequencies of the categorized uncertainties were recorded according to their specific location (e.g., data quantity, model relevance, model structure). The study findings suggest that systematic identification and categorization of uncertainty indicators, as guided by the framework, can help elucidate the specific locations where particular uncertainties exist in specific model predictions, such as whether the uncertainties mainly originate in the model structure, model performance, or is a question of mechanistic probability. It is concluded that the use of uncertainty indicators can support the identification of uncertainties, and the categorization framework has the potential to explicit-make implicit uncertainties, thus raising awareness among scientific communities and decision-makers about the uncertainties and assisting in the design of strategies to transparently communicate and address them.

P02

Non-Animal Preclinical Safety Testing for Pharmaceuticals

Jeffrey Brown, April Naab
PETA Science Consortium International e.V., Stuttgart, Germany

Abstract

New drugs are extensively tested on animals before they enter clinical trials, and only a few published case studies show examples of new therapies reaching humans with no prior animal studies. For all of the case studies, regulators and developers agreed on the absence of a pharmacologically relevant animal model and therefore selected a non-animal preclinical strategy as a last resort. With the goal of

changing this animal model default mindset, we developed an entirely non-animal preclinical safety testing strategy for our candidate therapeutic that prioritizes modern, human-relevant methods. Most regulators would argue that our candidate has an "appropriate" animal model, and our case study will provide the first example of avoiding animal studies in this situation. It will lay the groundwork for a much-needed shift away from the animal model default, towards the most scientifically-valid, human-relevant method available. We have discussed and refined our non-animal plan with both the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA). Both regulatory bodies agreed that the plan is reasonable, and the EMA even agreed that animal data is of "limited informative value" for our candidate.

The proposed poster will summarize existing published case studies of non-animal preclinical testing, and it will detail the non-animal plan developed for our candidate therapeutic. FDA Pre-IND meeting feedback and EMA Scientific Advice feedback will be discussed, and next steps focused on extending our candidate-specific regulatory feedback to *all* drugs in our candidate's same class will be explained.

P03

A Framework for Evaluating Mechanistic Evidence for Cancer Hazard Assessment: Read-Across Approaches to Evaluate Large Numbers of Chemicals

William Bisson[1], Whitney Arroyave[1], Sanford Garner[1], Gloria Jahnke[2], Alex Merrick[3], Ruth Lunn[3]
[1]Inotiv in support of NTP RoC, Research Triangle Park, USA. [2]AGOVx, Research Triangle Park, USA. [3]NIH/NIEHS/DTT, Research Triangle Park, USA

Abstract

The forthcoming update of the Handbook for Preparing the Report on Carcinogens (RoC) includes an expanded and more detailed section on mechanistic studies. This section provides transparency and guidance for using systematic review methods and evidence integration to reach a level of evidence (LoE) conclusion for carcinogenicity (i.e., convincing, supporting) from mechanistic studies.

Scoping and problem formulation activities are conducted to identify influential mechanistic questions: scientific issues that will most likely impact the LoE conclusions. When read-across is identified as an influential question for evaluation of substances with limited human and animal cancer data that share similarity with substances with cancer data, RoC will conduct further scoping activities to develop a read-across framework. The mechanistic section of the handbook provides high-level concepts for developing a framework, including scoping activities, developing a protocol, and conducting read-across evidence evaluation and integration. Based on scoping activities, the protocol will provide the hypothesis and elements in the prioritized read-across plan.

Once a plan is developed and the read-across has been conducted, evidence evaluation and integration are applied to the read across in three steps: (1) rating uncertainty of the predicted target chemicals based on quality of the cancer data for the sources, read-across argumentation, and similarity justification; (2) assessing the confidence in the read-across prediction; and (3) assessing and integrating the level of evidence.

We plan to apply these methods to several classes of chemicals under review for the RoC: the example of nitro-polycyclic aromatic hydrocarbons (NPAHs) will be highlighted in the presentation.

P04

Evaluation of Skin Sensitization Classification Rules to Reflect Induction Potency in Humans

Matthias Herzler[1], Kim T To[2], Aswani Unnikrishnan[2], Jaleh Abedini[2], David Allen[2], Dori Germolec[3], John Gordon[4], Nicole Kleinstreuer[5], Hon-Sum Ko[6], Joanna Matheson[4], Hermann-Josef Thierse[1], Jim Truax[2], Jens Vanselow[1], Judy Strickland[2]
[1]German Federal Institute for Risk Assessment, Berlin, Germany. [2]Inotiv, Research Triangle Park, NC, USA. [3]NIH/NIEHS/DTT/STB/NICEATM, Research Triangle Park, NC, USA. [4]U.S. Consumer Product Safety Commission, Rockville, MD, USA. [5]NIH/NIEHS/DTT/PTB/NICEATM, Research Triangle Park, NC, USA. [6]U.S. Food and Drug Administration, Silver Spring, MD, USA

Abstract

To support development of the Organisation for Economic Co-operation and Development's Guideline on Defined Approaches for Skin Sensitisation, we curated a human reference database of 2277 human predictive patch tests (HPPT) for 1366 unique substances. The United Nations Globally Harmonized System of Classification and Labeling of Chemicals (GHS) does not consider the number of sensitized subjects in HPPTs for human evidence in the classification as GHS subcategories 1A (strong, at least one subject sensitized at induction dose per skin area [DSA] 500 µg/cm2 or less) or 1B (other than strong, at least one subject sensitized at induction DSA greater than 500 µg/cm2). Using the human reference database, we applied a modified GHS approach to the extrapolated DSAs at which one individual is sensitized (DSA1+) or 5% of individuals are sensitized (DSA05). DSA, DSA1+, and DSA05 classified 605 test substances as sensitizers and 1650 as not classifiable. DSA, DSA1+, and DSA05 subcategorized 59, 208, and 182 test substances as GHS 1A, respectively. We used the DSA+1 statistic to evaluate reproducibility and concordance between human and animal reference data for binary classification and GHS subcategorization. The modified GHS approach allows consideration of induction potency in humans while providing good reproducibility and concordance with animal reference data. 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.

P05

Curating Chemical Use Categories and Exposure Predictions to Inform Chemical Assessment

Victoria Hull[1], Tripp R. LaPratt[1], Agnes L. Karmaus[1], Aswani Unnikrishnan[1], David G. Allen[1], Nicole C. Kleinstreuer[2]
[1]Inotiv, RTP, USA. [2]NIH/NIEHS/DTT/PTB/NICEATM, RTP, USA

Abstract

To contextualize chemical risk, it is essential to understand how human populations interact with and are exposed to chemicals from various sources. Exposure simulations and chemical use models can inform exposure scenarios for data-poor chemicals, but the large data volumes associated with these tasks can be difficult to navigate. To provide easily interpretable and accessible exposure and use data, we integrated predictions from the U.S. Environmental Protection Agency's (EPA's) SEEM3 exposure prediction models and use categories from EPA's Chemical and Product Database (CPDat) into the Integrated Chemical Environment (ICE; https://ice.ntp.niehs.nih.gov/). Population-level estimates of exposure were obtained from SEEM3 for chemicals within the model's applicability domain. Exposure estimates were annotated as "near-field" or "far-field" based on predicted exposure pathways. Chemical use categories in ICE were expanded to include functional use data from CPDat, which describes the roles chemicals serve within products. Functional use terms for nearly 9,500 chemicals were harmonized to categories established by the Organsation for Economic Co-operation and Development based on suggested synonyms and expert opinion. To characterize potential use for over 100,000 chemicals that lacked reported functional use, we added predicted functional use from CPDat. Presented alongside other toxicologically relevant data, this highly curated data will provide users with added context for evaluating chemicals. Further development of ICE will integrate chemical structure classifications using the Classyfire chemical taxonomy from the Wishart Research Group, allowing examination of associations between chemical structures with different use and exposure scenarios. Project was funded by NIEHS, Contract No. HHSN273201500010C.

P06

A Standardized Framework to Support FDA Qualification of Complex in Vitro Models for Use in Regulatory Submissions

Graham Marsh
Critical Path Institute, Tuscon, USA

Abstract

Complex in vitro Models (CIVM) are useful tools that can replicate human physiology in an in vitro system and promise to reduce animal use and improve preclinical safety testing. To define the components for a standardized framework to support FDA qualification of CIVM for use in regulatory submission, C-Path is hosting a public meeting with stakeholders for collaborative discussion on the Optimization of Models, Biomarkers and Validation, Model Performance, and Defining the Context of Use in order to identify the best path forward to qualify a liver CIVM. We aim to develop an evidentiary framework that includes identification of assessments and other standards, quality performance criteria, best practices, and reproducibility of complex in vitro models for use in regulatory decision-making and then apply this framework to test and initiate qualification of a context of use for a liver CIVM in regulatory decision-making. A team of stakeholders will summarize the recommendations and consensus from the public meeting to draft a white paper that addresses identification of assessments and other standards, quality performance criteria, best practices, and reproducibility of complex in vitro models for use in regulatory decision-making.

P07

Making Safety Decisions for a Sunscreen Active Ingredient Using Next Generation Risk Assessment: Benzophenone 4 Case Study

Matthew Dent[1], Sophie Cable[1], Nicola Hewitt[2], Jade Houghton[1], Hequn Li[1], Joe Reynolds[1], Predrag Kukic[1], Sharon Scott[1], Sophie Malcomber[1], Reuben Mascarenhas[3], Camilla Alexander-White[4], Maria Baltazar[1]
[1]Unilever, Bedford, United Kingdom. [2]Cosmetics Europe, Brussels, Belgium. [3]The Estée Lauder Companies Inc., London, United Kingdom. [4]MKTox Consultancy, Milton Keynes, United Kingdom

Abstract

Although an increasing number of safety assessments for systemic toxicity without using any animal data are becoming available, there are few examples of next generation risk assessment (NGRA) being used to address the systemic safety of an ingredient of regulatory interest, such as a UV filter. In this work, we conducted an exposure-led and hypothesis driven safety assessment of a UV filter (Benzophenone-4) used at an inclusion level of up to 5% in sunscreen products, based on the International Cooperation on Cosmetics Regulation principles of NGRA. The overall hypothesis was that if biological activity measured using a broad suite of human-relevant test systems is not observed at concentrations experienced systemically by sunscreen users, there can be no adverse effects associated with product use. To test this, experiments and computational modelling were conducted to i) provide a predicted consumer systemic exposure concentration of Benzophenone-4, to compare with ii) point(s) of departure obtained using human-relevant NAMs (cell stress, transcriptomics, in vitro pharmacological profiling) which provide information on bioactivity of Benzophenone-4. Because physiologically-based kinetic modelling indicated that concentrations of Benzophenone-4 would be higher in the kidney than in any other organ, bioactivity characterisation also included a primary human renal proximal tubular cell model. The safety decision was made based on the Bioactivity:Exposure Ratio and showed that no significant bioactivity would be expected in the human body at relevant exposures. In summary, this case study demonstrated that NGRA is a protective and useful approach for the safety evaluation of this UV filter.

P08

Considerations for Chemical Screening Using Behavioral Profiling in the Planarian Dugesia Japonica: Understanding the Impact of Experimental Parameters

Danielle Ireland[1], Elizabeth Rosenthal[1], Christina Rabeler[1], Zane Meyer[1], Winnie Lin[1], Kevin Bayingana[1], Gustav Allotey[1], Charisma Hasan[1], Eva-Maria Collins[1,2]
[1]Swarthmore College, Swarthmore, USA. [2]University of Pennsylvania, Philadelphia, USA

Abstract

The planarian species Dugesia japonica is a promising model for predicting neurotoxicity and developmental neurotoxicity. Rapid behavioral screening in multi-well plates can be used to assay the

effects of chemicals on neuronal function and development. Unique to this system, researchers can directly compare effects on adult vs regenerating/developing organisms to identify development-specific effects using a variety of readouts. Developing planarians can be screened into adulthood, which is reached within 12 days. Automated screening and data analysis using modern computational approaches promise robust results. However, to date, there has been no systematic study investigating how the experimental conditions, which may vary between laboratories, impact the results. We screened two solvents at multiple concentrations, and assay positive and negative controls at a single concentration in adult and regenerating D. japonica. We varied experimental conditions, including exposure duration/screening day, multi-well plate format (24 vs 48 vs 96-wells), and planarian culture conditions (static vs flow, circadian rhythm) and determined concordance across the conditions on different levels, from bioactivity identification to potency. We also evaluated the robustness of the different assays and endpoints by assaying how activity in specific endpoints was affected by these experimental parameters. This analysis will help identify the endpoints that provide the most robust information on chemical activity that should be used in future screening. This work provides a critical evaluation of the robustness of planarian screening by delineating how common sources of experimental variation impact the results. It also provides a framework for standardizing screening conditions across laboratories.

P09

Avoiding Acute Oral Toxicity Testing in Animals Through the Use of In Silico and In Vitro Information

Mark D Nelms[1], Bethany Cook[1], David Hines[1], Virginia K Hench[1], Jessica Ponder[2], Stephen W Edwards[1], Elizabeth Baker[2]
[1]RTI International, RTP, USA. [2]Physicians Committee for Responsible Medicine, Washington, D.C., USA

Abstract

Adverse outcome pathways (AOPs) can facilitate the transition to New Approach Methodologies (NAMs) by providing a scaffold to understand how mechanistic key events connect to apical adverse outcomes. However, for acute systemic toxicity, a network of AOPs are involved. In vitro methods that inform on toxicity mechanisms can provide insight into the complexity of key events leading to acute lethality. Toward this end, we used chemical structural similarity to group 11,992 chemicals with curated rat oral acute toxicity data. We algorithmically assigned a minimal assay set necessary to span all acutely toxic chemicals in each cluster; 98% of clusters required two or fewer assays. Additionally, chemicals were assigned a "final" AC50 value using the lowest AC50 among the cluster-specific minimal assay(s) and the chemical-specific cytotoxicity point. Cytotoxicity point values were used when AC50s for the minimal assay(s) were not available or exceeded cytotoxicity. When combining the structure-based clusters with activity from the associated minimal assays, the GHS classifications showed significant association. Results are promising that a combination of bioactivity and structural information may be as reproducible as traditional in vivo studies. To promote collaboration and facilitate evaluation of the utility of our structural similarity approach combined with prospective in vitro testing, we have created a workflow to identify the cluster a (confidential) chemical of interest fits within and the associated minimal assay(s). This will allow users to identify the most relevant in vitro testing battery to evaluate acute systemic toxicity of active ingredients based on chemical structure.

P10

NURA Offers Free New Approach Methodology Training on PBPK, Carcinogenicity, Pyrogenicity, PFAS and DASS in 2023

Eryn E Slankster-Schmierer
Physicians Committee for Responsible Medicine, DC, USA

Abstract

Regulatory agencies and chemical developers cite training as an important consideration for moving away from animal testing toward New Approach Methodologies (NAMs). The NAM Use for Regulatory Application (NURA) program responds to this growing demand by offering virtual and in-person NAMs training. Between May 2022 and April 2023, NURA hosted 18 webinars, providing 4,046 live training sessions to scientists and policymakers. All sessions are available free of charge at www.PCRM.org/NURA.

In this time, NURA held 4 multi-session training series featuring experts from regulatory agencies, academia, and industry. "DyNAMic Discussions: The Future is Already Here" was launched in collaboration with Unilever and the Institute for In Vitro Sciences, pairing two prominent experts on a targeted topic, offering their candid experience applying NAMs in a regulatory setting. Each session attracts 300 live attendees on average, and features an audience-led Q&A to address the barriers and solutions to increasing regulatory acceptance of NAMs by the users.

NURA launched "Human In, Human Out: Using Primary and Population Data for PBPK Analysis," training participants on applying computer models for regulatory assessment. A series titled "Tomorrow's Data Today: Sunsetting the 2-Year Carcinogenicity Assay" was launched with the goal of making the 2-year rodent carcinogenicity assay obsolete. Additionally, NURA hosted a series on pyrogenicity testing to inform toxicologists on alternatives to the traditional horseshoe crab and rabbit-based methods being used. On average, each session in these series trained over 200 live attendees.

NURA continues with plans for PFAS and skin sensitization NAM training into 2023-2024.

P11

An Exposure-Led Approach to Worker Safety Assessment of Sodium 2-Hydroxyethane Sulphonate Using New Approach Methodologies

Carl Westmoreland[1], Catherine Breffa[2], Caroline Chaine[3], Susann Fayyaz[2], Fabian Grimm[2], Steve Gutsell[1], Predrag Kukic[1], Reiko Kiwamoto[4], Moung Sook Lee[2], Colin Smith[5], Willemien Wieland[5], Adam Wood[1], Tristan Zellmann[6]
[1]Unilever, Bedford, United Kingdom. [2]Clariant Produkte Deutschland GmbH, Frankfurt am Main, Germany. [3]Vantage Specialty Chemicals, Ris-Orangis, France. [4]Unilever, Wageningen, Netherlands. [5]Environmental Resources Management Limited, Dordrecht, Netherlands. [6]Vantage Leuna GmbH, Leuna, Germany

Abstract

Next Generation Risk Assessment (NGRA) is an exposure-led approach to safety assessment that uses New Approach Methodologies (NAMs). The scientific principles of NGRA were applied to assure the safety of workers in factories handling sodium 2-hydroxyethane sulphonate (sodium isethionate, SI).

The worst-case levels of exposure of workers to SI in several factory environments were estimated using factory-specific data and occupational exposure models. These exposure values were then used to estimate levels of systemic exposure to SI following occupational exposure using Physiologically Based Kinetic (PBK) modelling. Experimental ADME data from NAMs were also generated on SI for this PBK modelling which indicated a worst-case plasma Cmax of 0.8 µM across the entire life cycle of SI.

The bioactivity of SI was assessed in a battery of NAMs relevant to systemic, reproductive, and developmental toxicity. Concentration-response curves were derived for 40 cell stress markers and High Throughput Transcriptomics was conducted in HepG2, HepaRG and MCF7 cells. Pharmacological profiling of SI against 73 targets was conducted as well as specific assays relating to developmental toxicity (Reprotracker, devTOXqp). Points of Departure (PoDs) for SI in these assays ranged from 104-5044 µM.

Cmax values obtained from PBK modelling of occupational exposure to SI were compared to PoDs from the bioactivity assays to derive Bioactivity/Exposure Ratios (BER) which demonstrated the safety for workers exposed to SI. This work provides additional evidence to support the application of NGRA for regulatory purposes such as REACH.

P12

Safety Assessment of Monographed OTC Cold/cough Medicine Using an *in Vitro* Testing Platform Based on Human Reconstructed Oral Tissues

Heather Walker[1], Emma Lear[2], Jakeb Phillips[2], Megan Creelman[2], Emily Acquaviva[2], Gertrude-Emilia Costin[2]
[1]Bayer Healthcare, LLC, Whippany, USA. [2]Institute for In Vitro Sciences, Inc. (IIVS), Gaithersburg, USA

Abstract

Over-the-counter (OTC) products are available to alleviate concurrent symptoms of colds and flu. They are primarily based on a combination of decongestants, antitussive and alpha adrenergic agonists which are well-established pharmaceutical agents covered by US monographs. Many of the active components of the OTC cough/cold drugs are bitter and must be masked using flavoring agents. Bayer Healthcare LLC employed internally a stringent safety testing program for OTC cough/cold medicine line extensions that require the products to be held in the mouth for a short period using an innovative testing platform based on reconstructed oral tissues. A total of 7 OTC cough/cold products were tested using a screening approach in which the products were applied topically to the surface of reconstructed oral tissues (EpiOral™, MatTek Corporation, Ashland, MA, USA) for 2 hours, followed by evaluation of tissue viability (by MTT reduction method) and assessment of inflammatory cytokines IL-1α and IL-1β. The compositions tested were finished products and Active Pharmaceutical Ingredients (APIs), in liquid or

tablet forms and designed for children and adults use. Our experiments confirmed that the products were safe to use based on the endpoints investigated that indicated no induction of irritation or inflammation up to 2 hours. The adoption of this *in vitro* testing platform attests the applicability and reliability of the modern technologies that not only support industry's due diligence and reduction in animal testing, but also demonstrate the relevancy of such platforms to human exposure while providing fast, biologically relevant safety data.

P13

Policy Initiatives for Integrating New Approach Methodologies for Pharmaceutical Testing

Emily Anderson, Elizabeth Baker, Susan Howe
Physicians Committee for Responsible Medicine, Washington, DC, USA

Abstract

New approach methodologies (NAMs) that utilize human cells and tissues (*in vitro*) and human data (*in silico*) for nonclinical safety and efficacy testing are increasingly being investigated by pharmaceutical companies to improve predictivity and efficiency, and reduce costs of drug development. Drug development stakeholders, including industry, regulators, and Congress, have conveyed the need to integrate NAMs into decision-making to deliver better therapeutics that improve human health while reducing and replacing animal use. However, animal tests are ingrained in regulatory policy and industry practice. To accelerate the development and adoption of NAMs, it is crucial that policies are modernized to provide regulatory certainty about NAMs use for meeting regulatory requirements and expectations.

Last year, the US Food and Drug Administration announced its plans to launch a cross-cutting New Alternative Methods Program (NAMP) aimed at advancing the development, qualification, and implementation of NAMs for regulatory use, which Congress funded at $5 million. As a core part of this initiative, the regulatory framework for pharmaceuticals should be updated to provide stakeholders with confidence to use NAMs in regulatory submissions, as many regulations require animal data and many guidance documents recommend animal tests. To account for ongoing NAMs progress, regulatory language should be changed to replace references to *animal* data requirements with *nonclinical* data requirements, clearly articulating the option to use NAMs for meeting regulatory requirements. Guidance documents should reflect practical flexibility for NAMs use and encourage NAMs use whenever possible.

P14

Weight of Evidence Toxicological Assessment of UV Filters Complemented by a NAMs Approach

Kimberly Norman[1], Carl D'Ruiz[2], Gary Eichenbaum[3], Alexandra Kowcz[1], J F. Nash[4]
[1]Personal Care Products Council, Washington, DC, USA. [2]dsm-firmenich, Parsippany, NJ, USA. [3]BioConvergent Consulting, Purchase, NY, USA. [4]The Procter and Gamble Company, Mason, OH, USA

Abstract

A comprehensive weight of evidence (WoE) assessment was performed using available toxicologic data for seven UV filters, avobenzone, ensulizole, homosalate, octinoxate, octisalate, octocrylene, and oxybenzone. Comprehensive dossiers for each UV filter were assembled using all available sources including REACh and NTP and contain data on pharmacokinetics, acute and repeat dose toxicity, genotoxicity, carcinogenicity, developmental and reproductive toxicity (DART), immunotoxicity, neurotoxicity, endocrine effects, and local effects, e.g., skin sensitization, skin irritation, photoirritation, and photoallergy. Importantly, these UV filters have no structural alerts for DNA reactivity and are non-genotoxic in standard *in vitro/in vivo* studies. These data were used to establish a point of departure for each UV filter and the margins of safety were high. New Approach Methods (NAMs) have the potential to provide a complementary approach for evaluating potential mechanisms and exposure levels at which chemicals may affect toxicological endpoints such as carcinogenicity and DART. We have developed a NAMs approach that includes evaluating the bioactivity of each UV filter and their major metabolites using cell-based assays in which dose-responses for receptor binding, immunomodulation, and cytotoxicity/proliferation are evaluated. The concentrations at which bioactivity is observed in these NAMs assays can then be compared to human plasma concentrations of the UV filters to evaluate whether there is a need to refine the point of departure. In conclusion, a WoE toxicological assessment of existing data supplemented by NAMs provides a robust approach to evaluate these UV filters and guide any potential refinements without the need for any new animal testing.

P15

Transcriptomic Read-Across for Characterization of Chemical Mechanisms-of-Action

Deepak Mav[1], Austin Ross[1], Michele Balik-Meisner[1], Dhiral Phadke[1], Scott S Auerbach[2], B. Alex Merrick[2], William M Gwinn[2], Ruchir Shah[1], Mike DeVito[3]
[1]Sciome LLC, Research Triangle Park, USA. [2]National Institute of Environmental Health Sciences (NIEHS), National Toxicology Program (NTP), Research Triangle Park, USA. [3]United States Environmental Protection Agency (US EPA), Research Triangle Park, USA

Abstract

To extend the capability of standard differential gene expression and pathway analysis, we have deployed a novel approach for characterizing mechanisms-of-action (MOA) of toxicological chemicals involving network analysis. A rank based statistical approach is used to calculate similarity between a query expression signature and signatures derived from other chemical/perturbagen transcriptomic experiments, enabling MOA predictions and transcriptomic read-across (TRA). As a case study, we evaluated liver TempO-Seq S1500+ data from rats exposed to the common environmental pollutant, perfluorooctanoic acid (PFOA). We first compared the PFOA expression signatures to those of 19 other chemicals assayed simultaneously. Results indicated PFOA bears mechanistic similarity with the peroxisome proliferator-activated receptor (PPAR) agonists fenofibrate, di(2-ethylhexyl)-phthalate, and triclosan; and the pregnane-X receptor activator tris(1-chloro-2-propyl)-phosphate. We then queried a collection of highly curated rat gene expression signatures compiled from analysis of public data such as TG-GATES and DrugMatrix. These signatures include varied perturbagens, organ/tissue systems, exposure durations, and concentrations. The PFOA query signatures clustered near database signatures

for fenofibrate, DEHP, and other PPARα-activating chemicals. Notably, dose-dependence was observed in the spatial positioning of the PFOA signatures for the eight study doses, with higher dose signatures being progressively closer to the database signatures of PPAR-activating chemicals. The findings verify the hypothesis for primary MOA of PFOA and further suggest roles for less well-established MOAs of PFOA. Our results serve as a proof-of-concept for characterizing the MOAs of chemical toxicants and facilitates comprehensive synthesis of transcriptomics data using novel bioinformatics inference. Additionally, interactive 3D visualization makes TRA easy and time-efficient.

P16

Applicability of NAMs for Skin Sensitization to Agrochemical Active Substances

Arthur Grivel[1], Elizabeth Webb[2], Christina Stahl[3], Helen Tinwell[1]
[1]Bayer SAS Crop Science, Sophia Antiopolis, France. [2]Bayer US Crop Science, Chesterfield, USA. [3]Bayer AG Crop Science, Monheim, Germany

Abstract

Traditionally assessed in guinea pigs or mice according to the OECD Testing Guidelines, substantial efforts towards non-animal methods have been made on skin sensitization in recent years. Mechanistic understanding of skin sensitization has resulted in the set-up of an adverse outcome pathway (AOP) and enabled the development as well as the validation of in silico/chemico/vitro assays, each relative to a specific key event (KE) (OECD, 2012).

Used in an integrated testing strategy validated in June 2021 under the OECD Testing Guideline n°497, these non-animal assays demonstrated a superior performance (88%) to the golden standard mouse Local Lymph Node Assay (LLNA) (58%) when compared to human data. This superior performance of the non-animal assays is due to a low specificity (22%), or true negative rate, of the LLNA to Human.

Primarily validated by OECD using cosmetic ingredients, the stand-alone KE new approach methodologies (NAMs) are de facto considered applicable to the broad chemical substances, including agrochemical small molecules, based on their physico-chemical properties. Being the first validated Defined Approaches (DAs) using NAMs and translated into a Testing Guideline by OECD, it is nonetheless helpful to provide the scientific community as well as regulatory authorities with case studies on the applicability of those methods and to build confidence in their utility in decision making. Therefore, the objective was to assess the applicability of NAMs for ten agrochemical active and compared to their historical animal data.

P17

Multi-Endpoint Acute Toxicity Assessment of Organic Compounds Using Large-Scale Machine Learning Modeling

Amirreza Daghighi[1], Gerardo M Casanola-Martin[2], Bakhtiyor Rasulev[2,1], Kweeni Idouku[1]
[1]Biomedical Engineering Program, North Dakota State University, Fargo, USA. [2]Department of Coatings and Polymeric Materials, North Dakota State University, Fargo, USA

Abstract

In recent years, alternative animal testing methods such as computational and machine learning methods, have become increasingly crucial for toxicity testing as they allow for minimizing animal examination while reducing costs and time. Machine learning is a powerful tool for in silico discovery in drug development and environmental chemical screening. However, the complexity and scarcity of available biomedical data challenge the development of predictive models. A combined approach of state-of-the-art non-linear machine learning methods and multi-condition descriptors offers a solution for combining data from various assays to create a more accurate and robust model. This study uses multi-condition descriptors (MCDs) to develop a QSTR (Quantitative Structure-Toxicity Relationship) model based on a large toxicity dataset of over 80,000 compounds and 59 endpoints, leading to 122,572 data points. The study discusses the prediction capabilities of developed seven single-task multi-endpoint machine learning models and a novel method of data analysis using Convolutional Neural Networks (CNN) to develop QSTR models. The results show that using MCDs significantly improves the predictability power of the model and using them with CNN-1D yields the best results (R^2=0.93, R^2_{ext} =0.70). Several structural features, such as van der Waals surface area, number of nitrogen-containing fragments, presence of S-P fragments, ionization potential, and presence of C-N fragments, showed the highest contribution to toxicity. These models can be useful tools for predicting the toxicity of various compounds under different conditions, enabling quick toxicity assessments of new compounds, and understanding their environmental impact.

P18

Advancing Animal-Free Environmental Safety Assessments of Cosmetics

Amelie Ott[1], Glauco Battagliarin[2], Marlies Bergheim[3], Arnaud Boivin[4], Bruno Campos[5], Iain Davies[6], Juliet Hodges[5], Sascha Pawlowski[2], Harald Streicher[7]
[1]International Collaboration on Cosmetics Safety (ICCS), New York, USA. [2]BASF, Ludwigshafen, Germany. [3]Henkel, Duesseldorf, Germany. [4]L'Oréal, Paris, France. [5]Unilever, Sharnbrook, United Kingdom. [6]PCPC, Washington DC, USA. [7]Beiersdorf, Hamburg, Germany

Abstract

After use, cosmetic ingredients are often disposed of down the drain to sewage treatment plants or in some regions, directly released to the environment. Consequently, several tools exist to determine environmental exposure, fate and hazard of chemicals to identify their safe uses in cosmetic products.

However, with a changing societal, scientific and regulatory landscape aimed at addressing evolving environmental challenges, new approaches are needed to ensure continued ability to assess safety. For example, animal tests on cosmetics are being increasingly banned around the globe.

For some environmental endpoints, viable alternatives exist but for others, more need to be developed (such as for endocrine disruption). In addition, more fit-for-purpose exposure and fate methodologies are required to address increasing environmental challenges (such as for polymers and mixtures).

The new global not-for-profit research organization International Collaboration on Cosmetics Safety (ICCS) is working to advance a 21st Century environmental toolbox to address environmental challenges through research, education, and regulatory engagement. This poster will summarize following on-going environmental projects:

- Tiered modelling framework for direct release of cosmetic ingredients to aquatic environments with a focus on UV filters
- Experimental and in silico approaches for biodegradation/persistence assessment of polymers
- Weight-of-evidence persistence assessment tool (PAT- for details, see other ICCS poster)
- International standard to calculate biodegradability of cosmetic formulations
- Identification of challenges and strategies for broader regulatory acceptance of environmental non animal new approach methodologies
- Advancing development of an internationally recognised guideline for acute and chronic coral toxicity

P19

Persistence Assessment Tool (PAT): Implementing a Methodology for Data Quality Evaluation and Weight-of-Evidence in Persistence Assessments

Christopher Hughes[1], Megan Griffiths[1], Simon Cook[1], Dik van de Meent[2], John Parsons[2], Delina Lyon[3], Amelie Ott[4]
[1]Ricardo Energy & Environment, London, United Kingdom. [2]Association of Retired Environmental Scientists ARES, Amsterdam, Netherlands. [3]Concawe, Brussels, Belgium. [4]International Collaboration on Cosmetics Safety (ICCS), New York, USA

Abstract

Chemical persistence plays a key role in determining environmental exposure. In the regulatory context, it involves comparing chemical degradation half-lives to set criteria for different environmental compartments (water, sediment, and soil). Other information is also relevant for assessments (e.g., biodegradation screening tests, non-standard experiments, QSARs, field data, etc.), and should be considered following a weight-of-evidence approach. Implementation challenges remain in persistence assessments, particularly relating to guidance around the evaluation of data quality, and the weight-of-evidence determination. In addition, there are issues for substances whose properties render them difficult to evaluate using standard methods.

To address these challenges, a software tool – the Persistence Assessment Tool (PAT) – has been developed to support the evaluation of persistence under regulatory frameworks such as EU REACH. This tool provides clear guidance and structure to evaluate data quality, and a quantitative weight-of-evidence (qWoE) methodology to process the information input and calculate persistence conclusions in line with regulatory guidance. The PAT is applicable to all substance types and provides specific features to account for difficult and complex substances. Various options for customisation of the methodology are included to adapt assessments to specific regulatory frameworks and purposes. In addition a multimedia fate model, SimpleRisk4PAT, is included to optionally calculate overall persistence (POV), allowing for additional potentially important environmental fate processes to be taken into account. The PAT aims to support robust, consistent and transparent decision-making for persistence assessment. There is a need for stakeholder input to support further validation, consensus-building and uptake of the methodology.

P20

Development of Toxicity Assays for Bluegill Gill and Lake Sturgeon Gill Cell Lines

Nadia Carmosini[1], Kelly Gorres[2], Anne M Wood[1], Mayanga C Kapita[1], Gavin N Saari[1]
[1]US Geological Survey, La Crosse, USA. [2]University of Wisconsin - La Crosse, La Crosse, USA

Abstract

Resource managers desire chemicals to control aquatic invasive species that are selective and will minimize impacts on native species. Identification of such chemicals is reliant upon the evaluation of toxicity among target invasive species and non-target native species. Historically, toxicity assays were performed with whole organisms, but the costs associated with animal maintenance, loss of life, and the production of large amounts of chemical waste are of increasing concern. To address these challenges, in vitro toxicity assays are being developed that use cell lines instead of whole organisms. This project is adapting the toxicity assay described in the Organisation for Economic Co-operation and Development (OECD) Test Guideline 249 for gill cell lines developed for two fish species native to North America, the Bluegill (*Lepomis macrochirus*) and Lake Sturgeon (*Acipenser fulvescens*). This assay has the potential to be utilized as a comparative toxicology tool for initial screening of chemical control candidates against invasive species for off-target effects on native species.

P21

Comparative Toxicity Assessment of Glyphosate and Two Commercial Formulations in the Planarian *Dugesia Japonica*

S. Grace Fuselier[1], Danielle Ireland[1], Nicholas Fu[1], Christina Rabeler[1], Eva-Maria S Collins[1,2]
[1]Swarthmore College, Swarthmore, USA. [2]University of Pennsylvannia, Philadelphia, USA

Abstract

Glyphosate is a widely used, non-selective herbicide. Glyphosate and glyphosate-based herbicides (GBHs) are considered safe at allowed environmental exposure levels. However, their growing usage has

triggered questions about possible adverse outcomes due to low dose chronic exposure. While the toxicity of GBH mixtures has primarily been attributed to glyphosate, other largely unstudied components of GBHs may be toxic or interact with glyphosate. We performed a comparative screen of glyphosate and two GBHs at the same glyphosate acid equivalent concentrations via behavioral phenotyping in the planarian *Dugesia japonica* to parse out their respective toxicity. Adult and regenerating planarians were screened to detect developmentally selective effects. Both GBHs were more toxic than pure glyphosate. While pure glyphosate induced lethality at 1 mM and no other effects, both GBHs induced lethality at 316 µM and sublethal behavioral effects starting at 31.6 µM in adult planarians. These data suggest that glyphosate alone is not responsible for the observed toxicity of the GBHs. Additionally, screening of the equivalent concentrations of the other active ingredients in these GBHs (diquat dibromide and pelargonic acid, respectively) revealed that GBH toxicity could not be explained by the active ingredients alone. Because all compounds induced toxicity at concentrations above environmental levels, our data indicates that glyphosate/GBH exposure is not an ecotoxicological concern for *D. japonica* planarians. Together, these data demonstrate the usefulness of high throughput screening in *D. japonica* planarians for assessing various types of toxicity, especially for comparative studies of several chemicals and mixtures across different developmental stages.

P22

Tri-Culture Human Gut Model for Microplastic and Nanoplastic Absorption Testing

Robert Gutierrez[1], Ana Barrios[2], Stephanie Cao[3], Elijah Petersen[1]
[1]NIST, Gaithersburg, USA. [2]GAO, Dallas, USA. [3]Montgomery College, Montgomery, USA

Abstract

Microplastics and nanoplastics (MNPs) have become a major environmental concern due to their volume and persistence. Data regarding the risks of MNPs to humans are still limited and is needed for improved hazard assessment. Ingestion is expected to be a key exposure routes in humans. There are, however, minimal studies on how these plastics affect the intestinal barrier. Therefore, there is an interest in creating a consistent in vitro model that is applicable and reproducible for risk assessments or MNPs. Therefore, a tri-culture model (tissue) composed of human intestinal epithelial (caco-2 and HT-29) cells and lymphocyte (Raji) cells as they are heavily used as an intestinal barrier. Measurements are taken periodically to check the integrity of the models by using transepithelial electrical resistance (TEER). These tissues are culture for 21 days and then exposed to gold nanparticles or polystyrene MNPs for 48 h to evaluate paracellular transport, TEER, and cell viability.

P23

Predicting Systemic Toxicity for Impurities From Medical Devices With (Q)SAR and Read-Across

Svava Osk Jonsdottir[1], Carsten B. Senholt[2]
[1]insilTox ApS, Kgs. Lyngby, Denmark. [2]SAXOCON A/S, Virum, Denmark

Abstract

The need for speed in risk assessment of chemical substances and materials urges the development, validation, and regulatory acceptance of New Approach Methodologies (NAM) as alternative to traditional costly and time-consuming animal studies [1]. In silico technologies play an important and still increasing role in NAM approaches under several regulations, including REACH, ICH M7 and the latest ISO 10997-17:2023 standard for toxicological risk assessment of medical device constituents. This can either be as a combination of in vitro and in silico methods, or as high quality in silico modelling and data integration.

Read-across analysis has shown to be the preferred method for obtaining estimates of point of departures (PODs) for risk assessment purposes, in cases where no data are available for the compound of interest. In recent years, much research effort has been devoted to developing better frameworks for grouping compounds and for identifying adequate source (analogue) compounds for read-across analysis [2].

Drawing on our experience in consultancy, this poster discusses estimation of POD values for systemic toxicity endpoints. The focus is on data gap filling for repeated-dose, developmental and reproductive toxicity for impurities, such as leachables and extractables from medical devices.

Through examples, it is illustrated how adequately performed read-across analysis, assisted by (Q)SAR, provides a framework for predicting reliable estimates for POD values. The potential, pitfalls and uncertainties associated with such analysis are discussed as well.

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2. Alexander-White, C. et al. Regul. Toxicol. Pharmacol., 2022;129:105094.

P24

Use of Alternative Vehicles in the h-CLAT to Expand its Applicability Domain and Analyze Chemical Mixtures

George L DeGeorge, Christopher T. Kalimtzis, Jutta Marzillier, Matthew L. Bobiak
MB Research Laboratories, Spinnerstown, USA

Abstract

Research efforts have been made to develop and validate in vitro tests for skin sensitization, aiming to replace the use of animals. Expanding the applicability of the h-CLAT to include mixtures has become highly desirable. Previously, we validated the h-CLAT to assess the skin sensitization potential of a limited number of commercially available mixtures. However, due to the diverse chemical composition of these products, it is necessary to explore alternative vehicles beyond those listed in OECD TG 442E to analyze chemicals and mixtures that are challenging to dissolve. We prioritized potential additional vehicles based on LogP values and assessed their compatibility with the assay using proficiency chemicals identified in OECD 442E. We conducted tests on several sensitizing and non-sensitizing mixtures available in the market (based on Safety Data Sheets). Our findings demonstrate that specific

alternative vehicles can provide better sensitivity and/or positive predictivity, when compared to established or traditional vehicles when testing mixtures. Building upon previous research, which indicated that acetone and 2-butanone are acceptable alternative vehicles for the h-CLAT, we show that these new vehicles can be successfully applied to assess the sensitization potential of both pure chemicals and mixtures. These results provide support for the development of multi-component vehicle systems for in vitro sensitization testing of mixtures, medical devices, and UVCB materials.

P25

Screening the Effects of Naphthenic Acid Contamination in the Athabasca Oil Sands Region Using an Avian 3D Spheroid Hepatic Cell Assay

Laura Van Raalte[1,2], Doug Crump[2], Lukas Mundy[2], Jason O'Brien[2]
[1]Carleton University, Ottawa, Canada. [2]Environment and Climate Change Canada, National Wildlife Research Centre, Ottawa, Canada

Abstract

The Athabasca Oil Sands Region (AOSR) is a major source of oil for Canada and one of the largest bitumen extraction sites in the world. Bitumen extraction produces large volumes of waste called tailings, which have been shown to be toxic to various organisms due to the presence of naphthenic acids (NAs). There is concern that tailings containment ponds may leach into surrounding tributaries and wetlands. The objective of this study was to determine NA concentrations and in vitro bioactivity of extracts derived from passive samplers deployed in AOSR wetlands. NA concentrations were determined using liquid chromatography-tandem mass spectrometry. Bioactivity was determined in 3D spheroid-cultured chicken LMH cells based on cytotoxicity, EROD and gene expression assays. We detected elevated levels of NAs in wetlands close to tailings ponds compared to reference wetlands. None of the extracts reduced cell viability. Changes in gene expression were found using a chicken ToxChip PCR array, which contains a curated list of toxicologically relevant genes. Genes in several different pathways were found to be dysregulated, including xenobiotic metabolism, immune, and lipid homeostasis pathways. Next steps include determining EROD activity to evaluate CYP1A induction. Bioactivity results will be used to estimate the relative avian toxicity of the extracts. Further, bioactivity data will be compared to NA concentrations to determine if they can be used to help predict NA contamination. Ultimately, we aim to demonstrate the utility of a non-animal, in vitro screening approach to enhance environmental monitoring efforts in a priority Canadian ecosystem.

P26

Evaluation of Alternative Solvents for Classification of Skin Sensitizers in the Kinetic Direct Peptide Reactivity Assay (k-DPRA)

Jessica Colvin, Holly King, Emily Acquaviva, Kristen Hopkins, Jakeb Phillips, Mery Marimoutou, Kayla Cantrell, Argel Islas-Robles
Institute for In Vitro Sciences, Inc., Gaithersburg, USA

Abstract

Testing for skin sensitization is needed to understand the potential of a substance to elicit an allergic reaction. The Kinetic Direct Peptide Reactivity Assay (k-DPRA) (OECD 442C) is an *in chemico* method, validated and adopted by OECD, which investigates the first step in the adverse outcome pathway for skin sensitization. The k-DPRA measures the reaction rate (log K_{max}) of a substance towards a cysteine-containing peptide, which is then used to classify the potency of sensitizers. Test guideline OECD 442C states that test substances must be soluble at 20 mM in either acetonitrile or phosphate buffer. In this study several solvents were explored for their applicability in the k-DPRA and potential use for difficult to solubilize substances. Three k-DPRA proficiency chemicals with known in vivo classifications were chosen: 2,4-dinitrochlorobenzene (DNCB) (strong sensitizer), ethylene glycol dimethacrylate (EGDMA) (weak sensitizer), and 4-methoxyacetophenone (MAP) (non-sensitizer). The selected substances were found to be fully soluble at 20 mM in all tested solvents: Acetone (ACE), Methanol (MeOH), and Isopropanol (ISO). For DNCB, EGDMA, and MAP, the log K_{max} were all found to be in the correct range when solubilized in either ACE or ISO. Although the reaction rates were correctly predicted for DNCB and MAP when solubilized in MeOH, the assay controls did not meet the criteria for a valid test, indicating that MeOH may not be fully compatible with the high throughput of the assay. Overall, the results show that ACE and ISO may be good candidates as alternative solvents in the k-DPRA.

P27

Case Study Application of a Systematic Approach to Inventory and Interrogate Thyroid Hormone Network Information

Isabel A Lea[1], S J Borghoff[1], D Wikoff[1], S Fitch[1], J Urban[1], C Perry[1], N Choksi[1], J Britt[1], M Heintz[1], W Klaren[1], R Chew[2], S Edwards[2], R J Bever[3], K Hamernik[3], A B Kirk[3], S G Lynn[3], K J Markey[3]
[1]ToxStrategies, Asheville, USA. [2]RTI International, Research Triangle Park, USA. [3]U.S.EPA, Washington, DC, USA

Abstract

Global efforts to better characterize thyroid hormone pathways have resulted in a continuously expanding heterogenous literature base with over 1,000,000 articles. Herein, a systematic mapping approach involving computational methods to inventory thyroid-related biological events is described. Pulling from a broad literature corpus, two molecular initiating event (MIE) categories (thyroid hormone (TH) serum distribution and membrane transport proteins) were investigated with the objectives of 1) identifying potential candidate reference chemicals with a range of potencies for test method verification, and 2) development of new test methods. Data labels were developed as DistillerSRTM question-answer sets allowing for computational automation through machine learning and natural language processing. From a narrowed corpus of 1662 articles focused on these two MIE categories, titles/abstracts were labelled by humans and computers. Of these, more than 400 articles included administration of chemicals in mammalian in vivo or in vitro test systems and were further evaluated to identify potential reference chemicals that could be used for test guideline development. Mining at the full-text level identified assays that used immortalized cell lines or primary cells that could be developed into high throughput approaches to screen for perturbations of processes involving TH distribution and/or membrane transport proteins. This case study demonstrates the utility of incorporating computational data labeling for efficient mapping of multiple study characteristics and interrogation of multiple research objectives.

The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA.

P28

Calibration and Evaluation of PFAS Toxicokinetics and Implementation in a Community-facing Tool to Estimate Individual Serum Levels

Meghan T Lynch[1], Weihsueh Chiu[2], Claire Lay[3], Rachel Rogers[4]
[1]Abt Associates, Cambridge, MA, USA. [2]bDepartment of Veterinary Physiology and Pharmacology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, USA. [3]Abt Associates, Boulder, USA. [4]Centers for Disease Control and Prevention/Agency for Toxic Substances and Disease Registry, Altanta, USA

Abstract

Background: Tools to assess the potential body burden associated with drinking per- and polyfluoroalkyl substances (PFAS)-contaminated water may be helpful for public health assessments of exposed people, but existing studies have reported varying PFAS half-lives (T½).

Approach: We combined data from multiple studies and used hierarchical Bayesian methods to develop estimates of T½ and volume of distribution (Vd), along with their interindividual variability, for perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), perfluorononanoic acid (PFNA), and perfluorohexane sulfonate (PFHxS). We then implemented a browser-based suite of one-compartment toxicokinetic models that estimate serum levels based on these values. We ran simulations of individuals with known PFAS water and serum concentrations and compared the predicted serum PFAS concentrations to measured data to evaluate model performance.

Results: Posterior median (95% CI) estimates of T½ (in years) for the population geometric mean were 3.14 (2.69, 3.73) for PFOA, 3.36 (2.52, 4.42) for PFOS, 2.35 (1.65, 3.16) for PFNA, and 8.30 (5.38, 13.5) for PFHxS. Vd estimates ranged from 0.19 to 0.43 L/kg, which tended to be slightly higher than previously published estimates. The models accurately estimate individual-level serum levels for each PFAS for most adults, but somewhat overestimated serum concentrations for children. They present an improvement over existing tools and incorporate a background exposure term in the absence of data describing exposure from other pathways.

Disclaimer: The findings and conclusions in this presentation have not been formally disseminated by the CDC/ATSDR and should not be construed to represent any agency determination or policy.

P29

Predicting the Toxicity of Fullerene Derivatives Using Classification Machine Learning Models

Gerardo M Casanola, Madeleine Williams, Amirreza Daghighi, Bakhtiyor Rasulev
North Dakota State University, Fargo, USA

Abstract

The toxicity of carbon-based nano molecules gives important insight into application of the molecules. Fullerene and fullerene derivatives (FDs) have previously been shown to be highly customizable to many applications. In this work a Quantitative Structure Activity Relationship (QSAR) model has been developed to predict the toxicity of the FDs. The ability of twelve machine learning models to consistently classify the FDs as toxic or nontoxic was examined. Information on 169 FDs was obtained from a previous work. The end point of the model was binary, the FD is either toxic or nontoxic. 5,666 descriptors were calculated in alvaDesc, and a subset of important features was created using forward feature selection. External validation and AUROC were used to determine the most effective model. Internal validation was used to confirm the consistency of the model. The applicability domain was defined with the range, Euclidean distance, and probability calculated using Ambit Discovery software. Logistic regression was found to be the model with the highest accuracy, ACC = 98.6%. Three influential descriptors were found, ATSC8i, DP08 and CATS2D_04_DA. A physical understanding of the descriptors and correlation to the output of the model was determined. A virtual library of 41803 FDs was generated and then categorized with the QSAR model. With the results of this work, query FDs' toxicity can be predicted. Understanding of this prediction can be established from the formula for the logistic regression algorithm. Future manipulation of the molecule will be assisted by the physical representation of the descriptors.

P30

The Application of New Approach Methodologies (NAMs) for Next-Generation Tobacco and Nicotine Products Assessment

Kyeonghee Monica Lee[1], Liam Simms[2], Kei Yoshino[3], Marianna Gaca[4]
[1]Altria Client Services LLC, Richmond, USA. [2]Imperial, Bristol, United Kingdom. [3]Japan Tobacco Inc, Yokohama, Japan. [4]British American Tobacco, Southampton, United Kingdom

Abstract

The field of in vitro toxicology has accelerated in recent years with the advances in computational tools and human in vitro tissue systems. These New Approach Methodologies (NAMs) in cellular and molecular biology facilitate a paradigm shift in toxicity testing, harnessing mammalian cell lines of increased human relevance. These in vitro-based tools are implemented in chemical and candidate drug screening, also driven by the need for faster and clinically relevant toxicological assessment.

Alternative, next-generation tobacco and nicotine products (NGPs) including heated tobacco products, electronic cigarettes, smokeless tobacco products and tobacco-free oral nicotine pouches, have the potential to reduce the risk of chronic diseases compared to cigarettes. NAMs offer effective screening tools as part of testing framework for the assessment of such products and start to demonstrate utility in NGP development and evaluation.

CORESTA is an international organization, developing and promoting research on tobacco and nicotine products, with >800 experts from 162 organizations (industry, contract laboratories, academic, governmental and non-governmental organizations). In the last few years, CORESTA Next Generation

Tox Task Force and In Vitro Subgroup have explored the application of NAMs in tobacco regulatory sciences, through literature reviews, scientific studies, and recently at two annual symposiums. In this presentation, we share the goals and outcomes of these symposiums, the utilities and strengths of NAMs as well as gaps and opportunities and related CORESTA activities. The importance and opportunity for fit-for-purpose testing and method standardization will also be discussed to support the regulatory acceptance and implementation of NAMs for NGP testing.

P31

Physiologically Based Pharmacokinetic Modeling and Simulation of Cannabinoids in Human Plasma and Tissues

Yitong Liu, Robert Sprando
Food and Drug Administration, Laurel, USA

Abstract

There has been an increased public interest in developing consumer products containing nonintoxicating cannabinoids, such as cannabidiol (CBD) and cannabigerol (CBG). At the present time, there is limited information available on the pharmacokinetics of cannabinoids except CBD in humans. Since pharmacokinetic profiles are important in understanding the pharmacological and toxicological effects at the target sites, physiologically based pharmacokinetic (PBPK) modeling was used to predict the plasma and tissue concentrations of 17 cannabinoids in humans. PBPK models were established using measured (in vitro) and predicted (in silico) physicochemical and pharmacokinetic properties, such as water solubility and effective human jejunal permeability. Initially, PBPK models were established for CBD and the model performance was evaluated using reported clinical data after intravenous and oral administration. PBPK models were then developed for 16 additional cannabinoids including CBG, and the plasma and tissue concentrations were predicted after 30 mg oral administration. The pharmacokinetic profiles of the 16 cannabinoids were similar to CBD, and the predicted plasma concentration and time profiles of CBD agreed well with clinical data in the literature. Although low exposure was predicted in the plasma (maximum plasma concentrations < 15 nM), the predicted tissue concentrations, especially the liver (maximum liver concentrations 70–183 nM), were higher after oral administration of 30 mg cannabinoids. These predicted plasma and tissue concentrations could be used to guide further in vitro and in vivo testing.

P32

Web Application to Predict Skin Sensitization Using Defined Approaches

Kim T To[1], Judy Strickland[1], Emily Reinke[1], Alexandre Borrel[1], Jim Truax[1], David Allen[1], Nicole Kleinstreuer[2]
[1]Inotiv, Research Triangle Park, NC, USA. [2]NIH/NIEHS/PTB/DTT/NICEATM, Research Triangle Park, NC, USA

Abstract

Defined approaches for skin sensitization (DASS) have been developed to identify potential chemical skin sensitizers by integrating data from multiple non-animal tests using data interpretation procedures.

While certain DASS have been internationally accepted by regulatory agencies, the data interpretation procedures they use vary in logical complexity, and manual application can be time-consuming and error prone. We developed an open-source web application, the DASS App, to facilitate programmatic implementation of four DASS: the Two-out-of-Three (2o3), two versions of the Integrated Testing Strategy (ITS), and the Key Event 3/1 Sequential Testing Strategy (KE 3/1 STS). To predict skin sensitization hazard, the 2o3 is based on consensus among three tests; ITSv1 and ITSv2 implement a scoring scheme using three tests; and the KE 3/1 STS is based on a stepwise evaluation with two tests. ITS can also be used to predict potency categorization using criteria from the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS). The DASS App enables users to implement non-animal approaches to evaluate chemical skin sensitization without the need for additional software or computational expertise. The application supports upload and analysis of user-provided data and provides hazard and potency predictions in a downloadable format. The latest update introduced the ability to evaluate DASS predictions against user-supplied reference data. The DASS App is available online at https://ntp.niehs.nih.gov/go/40498. This project was funded with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.

P33

Using 3D Constructs for the Evaluation of an Oral Irritation Assay

Robert Gutierrez, Blaza Toman, John Elliot, Elijah Petersen
NIST, Gaithersburg, USA

Abstract

The testing of biocompatibility is often one of the final steps in the evaluation of dental materials. To both minimize the failure rate and the use of animal testing at this final stage, there is a need to use new approach methodologies (NAMs) such as in vitro models to preformed biocompatibility testing. Here we describe the assessment of an oral irritation assay by looking at the changes in the viability of commercially acquired 3D constructs (tissues) using the MTT assay. These tissues are more physiologically relevant as they mimic the human oral environment compared to 2D constructs. Using cause-and-effect analysis and flow charts we created a plate layout for this study. We evaluated key aspects such as negative controls, solvents (sesame seed oil and saline solution), positive controls (1 % Triton-X), uncertainty of pipetting, and repeatability. We also tested known irritants like Y-4 polymer and sodium dodecyl sulfate (SDS) (dentally relevant substances) at varying concentrations. With this information we were able to create a statistical model to support assay design and determine the assessment of test substances as yielding a positive or negative result.

P34

Comparative Analysis of Ames Test Outcomes Across Five OECD-Recommended Strain Groups and Their Various Subsets

Vijay K Gombar[1], Alexander Y Sedykh[1], Adrian J Green[1], Ruchir R Shah[1], Kristine W Witt[2], Warren Casey[2]
[1]Sciome LLC, Research Triangle Park, USA. [2]National Institute of Environmental Health Sciences (NIEHS), Division of Translational Toxicology (DTT), Research Triangle Park, USA

Abstract

The standard bacterial mutagenicity Ames test (OECD 471 guideline) is conducted in five bacterial strains both with and without exogenous metabolic fraction S9. However, there is an ongoing effort to maximize testing efficiency by using fewer strains. Here we report analyses of various multi-strain testing strategies by filling data gaps with QSAR predictions for partially tested Ames Negative compounds.

Available data with 80,778 records containing Ames test outcomes in individual strains (PubChem Assay ID 1259407) were cleaned and harmonized resulting in 6,286 compounds. QSAR models using three different modeling methods (Recursive Partitioning, Support Vector Machines, and Random Forest) were then developed for each of the five strain groups with and without S9. A total of 11,005 gaps for 2,438 partially tested Ames Negative compounds were filled with consensus predictions from these QSAR models.

Results: (a) any single strain detects at most around 60% of the Positives (TA100 64%; TA98 59%; TA1537 45%; E. coli 35%; TA1535 23%), (b) two-strain combinations, TA98+TA100 and TA1537+TA100 retrieve 83% and 81% of Positives, respectively, (c) the best three-strain combinations, detecting 92% of all Positives, are TA100+E. coli combined with either TA98 or TA1537, and (d) the best four-strain combination excludes TA1535 and retrieves 97% of Positives.

We conclude that the existing redundancy among strains may be taken advantage of by conducting a test using a reduced set of strains that give acceptable trade-offs in test accuracy vs efficiency. Structural features leading to Positive outcome in various Ames strains will be discussed.

P35

Deep Learning Profile QSAR Modeling to Impute In Vitro Assay Results and Predict Chemical Carcinogenesis Mechanisms

Alexandre Borrel[1], Agnes L. Karmaus[1], Getachew Tedla[1], Kamel Mansouri[2], Thomas Luechtefeld[3], Ruth Lunn[4], Amy Wang[4], Dave G Allen[1], Nicole Kleinstreuer[2]
[1]Inotiv, RTP, USA. [2]NIH/NIEHS/DTT/PTB /NICEATM, RTP, USA. [3]Insilica, Bethesda, USA.
[4]NIH/NIEHS/DTT/IHAB, RTP, USA

Abstract

Carcinogenesis is a multistep process in which normal cells acquire properties that allow them to form benign tumors or malignant cancers. These properties have been associated with 10 well-established hallmarks of cancer. The concept of key characteristics of carcinogens (KCC) has also been developed to describe 10 properties that are shared by viruses and chemicals that induce human cancers. Quantitative structure-activity relationship (QSAR) models that rely on structural or physicochemical properties to predict carcinogenesis potential endpoints usually perform poorly, likely because they lack sufficient information on the complex mechanisms involved in carcinogenicity. We combined a novel imputation profile QSAR modeling approach with modern machine learning to analyze data on 10,000 Tox21/ToxCast chemicals and 2,000 in vitro assay endpoints associated with KCC. Because limited

experimental data were available, we filled data gaps by imputing assay results for the Tox21/ToxCast inventory using structural and physicochemical properties and deep learning. Imputed in vitro assay results were enriched using data in the BioBricks platform, which compiles toxicity-relevant databases into a harmonized easily accessible format. This enrichment allowed us to include additional information such as protein target binding or assay results to the model. Finally, various machine learning approaches including a multitask deep learning model were applied to predict each chemical's likelihood of inducing cancer based on the imputed in vitro data. Results included output metrics on the quality of imputation, defined by grouping of assays, and performance computed per chemical. Project was funded by NIEHS under Contract No. HHSN273201500010C.

P36

Application of in Vitro Transcriptomics Point of Departure and IVIVE in Early Agrochemical Discovery Programs

Enrica Bianchi
Corteva Agriscience, Indianapolis, USA

Abstract

Recent efforts identifying alternatives to animal testing have focused on predictive and precision testing, with ample development of high-throughput screening, new technologies, and alternatives to animal testing. In particular, transcriptome-based in vitro approaches could provide a powerful tool for regulatory decision-making among with physiologically based kinetic (PBK) modelling that represents a well-established and accepted methodology for improving quantitative in vitro-to-in vivo extrapolation (IVIVE). Furthermore, while high-throughput in vitro toxicity screening provides an efficient way to identify potential biological targets, reliance on the nominal chemical concentrations in these in vitro assays as an indicator of bioactivity may misrepresent potential in vivo effects due to differences in clearance, protein binding, bioavailability, and other pharmacokinetic factors. PBK modeling provides an effective framework for conducting quantitative IVIVE to solve this dilemma. Given that previous findings suggested apical and transcriptomic endpoints show similar points of departure (POD) in vivo, we tested the hypothesis that an in vivo transcriptional POD could be accurately predicted from an in vitro POD using PBK modeling. Transcriptional profiles from rat liver samples and rat primary hepatocytes exposed to different chemicals from the TG-GATEs database were used to generate transcriptome PODs followed by PBK based IVIVE to estimate rat-relevant exposure. Our findings suggest a stronger correlation between in vitro and in vivo transcriptional PODs using measured plasma protein binding and hepatic clearance data when data were generated using appropriate in vivo dose selections. These findings suggest that PBK modeling improves the prediction of in vivo PODs from cellular-based transcriptome data.

P37

Scope and Financial Impact of Unpublished Data and Unused Samples Among U.S. Academic and Government Researchers

Emma C Bowers[1], Jimena Stephenson[1], Melissa Furlong[2], Kenneth Ramos[3]

[1]LabPair, Tucson, USA. [2]University of Arizona Mel and Enid Zuckerman College of Public Health, Department of Community, Environment, and Policy, Tucson, USA. [3]Texas A&M Institute of Biosciences and Technology, Center for Genomic and Precision Medicine, Houston, USA

Abstract

Efforts to reduce and refine the use of animals in research are limited by broader inefficiency and bottlenecks in the research process itself. For example, animal studies are needlessly repeated when data go unpublished. When non-target ancillary tissues are discarded and banked samples fail to find use, more studies are conducted than would otherwise be necessary. To obtain a greater understanding of these issues and their underlying causes, we conducted an anonymous survey study to estimate the extent of unpublished scientific data and unused samples. Responses were received from 301 academic and government scientists from a variety of fields.   Respondents estimated that they published ~60% of their data and 95% had unpublished data. Of those collecting specimens, 60% stored unused samples. Many respondents had tissues from their previous animal studies. Unfinished projects were the largest source of unpublished data, with systemic and logistical issues playing contributary roles. With regard to unused samples, knowledge of their existence or access to collaborators were the greatest obstacles to sharing. The median cumulative self-reported estimated value of unused resources per researcher was $28,857, with life science ($36k) and government ($109k) researchers reporting the costliest assets. Using NSF headcounts, we estimated that the current cumulative value of unused resources at universities is approximately $6.2 billion, representing ~7% of the current annual US R&D budget. These findings identified obstacles that undermine scientific progress and productivity and offer valuable insights to reduce and refine the use of animals in research and to realize significant resource savings.

P38

Facilitating NAM-Based Chemical Assessments with the Integrated Chemical Environment

Aswani Unnikrishnan[1], Victoria Hull[1], Xiaoqing Chang[1], Alexandre Borrel[1], Kimberly To[1], Agnes Karmaus[1], Jason Phillips[2], Eric McAfee[2], David Allen[1], Nicole Kleinstreuer[3]
[1]Inotiv, RTP, USA. [2]Sciome, RTP, USA. [3]3NIH/NIEHS/DTT/PTB/NICEATM, RTP, USA

Abstract

The National Toxicology Program's Integrated Chemical Environment (ICE, https://ice.ntp.niehs.nih.gov/) provides highly curated data and computational tools to facilitate the exploration, characterization, and interpretation of chemicals use, exposure, and hazard. ICE data and tools are frequently updated to address evolving stakeholder needs. The latest release introduced new datasets, chemical lists, and features that further enhance the capabilities of ICE tools. ICE users can now obtain population-level exposure predictions from the U.S. Environmental Protection Agency's SEEM3 prediction model through the ICE Search tool and the ICE REST API. Exposure estimates can also be compared to the equivalent administered doses predicted by the ICE In Vitro to In Vivo Extrapolation (IVIVE) tool. To support evaluation of new approach methodologies (NAMs) for developmental toxicity, the ICE Physiologically Based Pharmacokinetic (PBPK) and IVIVE tools now include a gestational model from the EPA's httk

package (v2.2.2). The release also revised the ICE Chemical Characterization tool, implementing updates to curated chemical product use categories and adding reported and predicted functional use categories. The ICE Search tool now houses a beta Query Summary results tab, which provides summary visualizations to help users contextualize and interactively explore the data based on their specific needs. The presentation will detail these features and demonstrate how ICE can provide user-friendly solutions to navigating complex data and tools as well as contribute to establishing confidence in applying NAMs for chemical assessments.

P39

Incorporating New Approach Methodologies Into a Tiered Assessment Framework for Agrochemical Metabolite Human Safety Assessment

Agnes Karmaus[1], Amber Goetz[1], Angela Hofstra[2], Garrett Morandi[2], Christopher Schlosser[1], Ted Valenti[1], Alexander Charlton[1]
[1]Syngenta, Greensboro, USA. [2]Syngenta, Guelph, Canada

Abstract

The application of new approach methodologies (NAMs) to address regulatory data requirements for agrochemicals is rapidly developing. Safety assessments, and more specifically estimates of human exposure are developed for both agrochemical active ingredients and their metabolites that may potentially form in the environment, crop, or livestock matrices. Current approaches for establishing toxicological reference values for metabolites are reliant upon structure-based grouping with limited application of read-across, which is challenging for the prediction of toxicological reference doses, and the conduct of time-intensive and low-throughput animal toxicology studies. The use of NAMs to address the safety of agrochemical metabolites presents a promising opportunity to move away from traditional approaches towards more targeted methods which can be more directly applicable to humans.

We propose a tiered assessment framework for determining agrochemical metabolite toxicity with the goal of focusing on 'fit for purpose' registration packages. By using knowledge from existing active ingredient studies supplemented with in vitro and in silico tools, this framework supports the use of grouping based upon commonality of biological perturbations. Investigating interactions with common molecular targets allows for toxicity profiles to be extrapolated on the basis of mode of action (e.g., biologically-based read-across). Our aim with presenting this framework is to encourage future dialogue among industry and between regulators to present scenarios by which implementing NAMs can be used to derive toxicological reference values for risk assessment whilst maintaining transparency and reproducibility.

P40

Compilation and Standardization of Rat Acute Inhalation Study Data to Support Predictive Modeling

Victoria Hull[1], Emily N. Reinke[1], Amber B. Daniel[1], Kimberly T. To[1], Agnes L. Karmaus[1], David G. Allen[1], Kamel Mansouri[2], Nicole C. Kleinstreuer[2]
[1]Inotiv, RTP, USA. [2]NIH/NIEHS/DTT/PTB/NICEATM, RTP, USA

Abstract

Computational models for predicting acute inhalation toxicity have been proposed as alternatives to animal tests to support regulatory decision making. Developing such models requires robust, well-curated, and chemically diverse training data. NICEATM has compiled and curated rat acute inhalation data for approximately 1200 chemicals from a variety of sources, including the European Chemicals Agency, the U.S. Environmental Protection Agency, the U.S. National Institute for Occupational Safety and Health, the U.S. Department of Defense, and PubChem/ChemIDPlus. Concentrations lethal to half the test animals (LC50) values and study metadata were extracted and evaluated for quality using manual and automated techniques. For data meeting predetermined quality standards, LC50 values were converted to 4-hour exposures using Haber's Law to facilitate direct comparison and hazard category assignments. For nearly 70% of studies, details were not available on whether the chemical was delivered via aerosol, vapor, or gas. For these chemicals, a rule-based decision process based on physicochemical properties was applied to assign the phase of exposure and associated hazard category. Data were analyzed for variability across categories for chemicals with multiple studies and exposure types. Nearly 200 chemicals had at least three LC50 point-estimates, and more variability was found between chemicals than within chemicals. The curated data will be made publicly available in NICEATM's Integrated Chemical Environment and used in a collaborative modeling effort to generate consensus predictions for continuous, binary, and multicategory endpoints for regulatory hazard assessment. This project was funded by NIEHS, NIH under Contract No. HHSN273201500010C.

P41

In Vitro Assessment of Cytotoxicity and Cytokine Release Using a 3D Buccal Tissue Model to Evaluate Flavor and Surfactant Changes in Clinically Tested Mouthrinses

Kimberly Reed[1], Emily Acquaviva[2], James McGuire[1], Andrew Glowacki[1], Mary Lynn Bosma[1], Patricia Gorecki[1], Rosemarie Wallace[1], Jakeb Phillips[2], Emma Lear[2], Megan Creelman[2], Gertrude-Emilia Costin[2]
[1]Kenvue Inc., Skillman, USA. [2]Institute for In Vitro Sciences, Inc., Gaithersburg, USA

Abstract

Ingredient concentration or flavor component changes could alter the oral tissue irritation potential of mouthrinses. The 3D buccal tissue model (BTM) is an in vitro model that has been previously described to assess mouthrinse impacts on tissue. A modified BTM method was developed to evaluate the irritation potential of such formula changes as a bridge to clinically evaluated formulations.

The BTM method uses serum free media and a tissue-air interface application of undiluted mouthrinse to evaluate the cell viability and cytokine release from the 3D tissue grown from normal human oral/buccal cells. The method was optimized to achieve 40-50% cell viability of the benchmark product that allows identification of non-inferiority of the formula changes as linked to clinical outcomes. Cell

viability is determined by the NAD(P)H-dependent microsomal enzyme reduction of MTT. Cytokines including IL-1α and IL-1β are released into the medium below the tissue allowing for quantification via ELISA.

This method has been utilized to evaluate 25+ flavors in multiple formula types. Inter-lab reproducibility has been observed through evaluating the benchmark mouthrinse in six separate studies at the internal sponsor lab and external partner lab. The results yielded cell viability of 45% and 40% respectively. These BTM cell viability trends were replicated clinically through assessments of oral exfoliation incidence.

BTM has shown ability to evaluate flavor change impact of mouthrinses: allowing identification of oral tissue irritation trends.

P42

A Novel Standardized In Vitro Islet Model System for Efficacy and Toxicity Testing in Pancreatic β-cells

Madhu Nag, Burcak Yesildag
InSpheroAG, Schleiren, Switzerland

Abstract

The lack of in vitro models that reliably assess pancreatic islet function for toxicity and efficacy testing remains a significant challenge in drug development. To address this unmet need, we have developed a standardized 3D islet microtissue model generated from dispersed islets and cultured in 96-well-plates in a single microtissues format. We observed robust glucose-dependent insulin secretion from islet microtissues across donors, with stable glucose responsiveness, viability and size during long-term culture, as well as homogenous size distribution, tissue architecture and cellular composition allowing high-throughput data acquisition with low intra-assay variability. To evaluate our model for compound risk assessment, human and rat islet-microtissues from individual donors were used to assess chronic effects of marketed drugs previously associated with pancreatic β-cell toxicity. Olanzapine (an antipsychotic drug), Tunicamycin (an ER stress-inducing antibiotic), Tacrolimus and Rapamycin (immunosuppressive agents), were evaluated in a dose-dependent manner for their influence on islet ATP and insulin content, chronic, basal and glucose-stimulated insulin secretion following 14 days of compound exposure. In human islet microtissues, Tunicamycin treatment resulted in decreased ATP (>1µg/ml) and insulin content (>0.1µg/ml), impaired chronic, basal and glucose-stimulated insulin secretion (>0.1µg/ml). Olanzapine decreased insulin content (>10µM) and impaired chronic insulin secretion (>10µM). Tacrolimus and Rapamycin suppressed chronic (>10nM and 0.1nM), basal (>0.01nM and 0.1nM) and glucose stimulated insulin secretion (>10nM and 1nM). Rat islet microtissues displayed similar trends in compound sensitivity for all compounds except Olanzapine. Robust long-term functionality of islet microtissues makes them an ideal model for in vitro assessment of drug-Induced pancreatic endocrine injury.