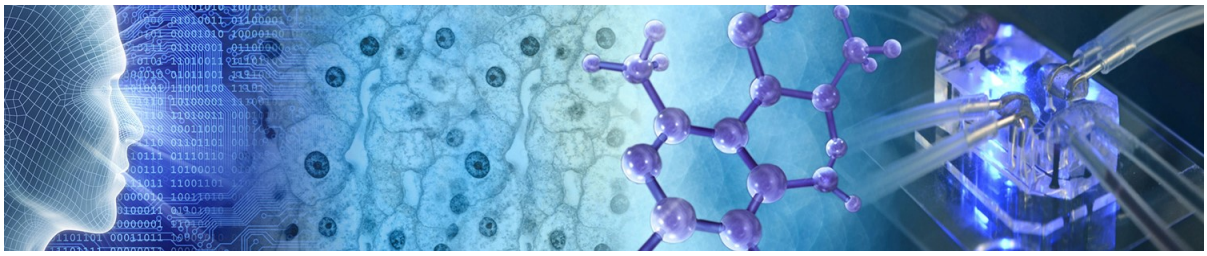
Biennial Progress Report 2018-2019

Interagency Coordinating Committee on the Validation of Alternative Methods



July 2020

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About this Report

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) facilitates the development, validation, and regulatory acceptance of test methods that replace, reduce, or refine the use of animals in testing.

The ICCVAM Authorization Act of 2000 directed ICCVAM to prepare a progress report on its first anniversary and biennially thereafter.

In January 2018, ICCVAM published A Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States. The roadmap describes how ICCVAM agencies will encourage development of new technologies for, support utilization of, and build confidence in new methods. This report summarizes progress toward these goals during 2018–2019.

The ICCVAM Biennial Report is prepared by the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), with support from the National Toxicology Program Office of Liaison, Policy, and Review.

Send comments or suggestions to NICEATM

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Message from NIEHS and NTP

In the 2018-2019 Biennial Report, we are pleased to share with you the many accomplishments of the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), and the ICCVAM agencies to address needs and priorities identified in the Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States. In particular, we highlight continued efforts to eliminate animal use for acute toxicity testing with important progress in skin sensitization assessment and an international partnership to develop the Collaborative Acute Toxicity Modeling Suite. Other efforts have leveraged alternative approaches such as high-throughput screening and computational tools to address the evaluation of complex endpoints including carcinogenicity, developmental toxicity, and neurotoxicity. Achievements noted at the U.S. Environmental Protection Agency (EPA) and the U.S. Food and Drug Administration (FDA) have further advanced alternatives to animal testing and reductions in animal use.

A key area of the Strategic Roadmap is communication and collaboration. We applaud the progress by NICEATM and ICCVAM to expand outreach efforts that progress the acceptance and use of alternative to animal testing. The many workshops, publications, and webinars have both raised awareness about areas with testing needs and communicated where testing approaches and solutions are available. This report highlights ICCVAM's many efforts to inform and connect with stakeholders through national and international partnerships that advance test method development, evaluation, and acceptance.

We want to highlight the recognition of ICCVAM's leadership by others. In 2018, ICCVAM Co-chair Anna Lowit, Ph.D., of EPA received the Society of Toxicology (SOT) Enhancement of Animal Welfare Award for her ICCVAM leadership and work at EPA's Office of Pesticide Programs to improve risk assessments while reducing animal use in toxicity testing. In 2019, Suzanne Fitzpatrick, Ph.D., FDA's principal representative to ICCVAM, received the SOT Enhancement of Animal Welfare Award that recognized her expertise in alternative toxicological methods and work to improve animal welfare within the field of toxicology.

As we look forward to continued progress, we note an organizational change at NICEATM. We welcome former NICEATM Deputy Director Nicole Kleinstreuer, Ph.D., as the new Acting Director of NICEATM. Nicole was honored in 2019 with the SOT Achievement Award for her leadership and scientific contributions to alternative toxicological methods and computational toxicology. Warren Casey, Ph.D., who served as NICEATM Director from 2013 to 2019, will remain ICCVAM Administrative Director. We want to thank Warren for his dedicated service and acknowledge his key roles in developing the Strategic Roadmap and fostering collaboration with ICCVAM's stakeholders.

We invite you to read the Biennial Report to learn more about all that's been accomplished in the past two years to advance alternative methodologies and the 3Rs.

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Message from NICEATM and ICCVAM

On behalf of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), we are pleased to present the 2018-2019 ICCVAM Biennial Progress Report. This report provides the most comprehensive overview ever of activities by ICCVAM and its 16 member agencies that support the ICCVAM mission. Published on the 20th anniversary of the ICCVAM Authorization Act, this report for the first time includes contributions from all 16 ICCVAM agencies describing their efforts to reduce, refine, and replace animal testing.

Our primary focus since the January 2018 publication of the Strategic Roadmap has been to implement the roadmap goals for acute systemic toxicity, skin and eye irritation, and skin sensitization. Recent ICCVAM activities advanced both development of new approaches for assessing these endpoints and regulatory initiatives to encourage use of non-animal approaches for acute toxicity testing.

This Biennial Report also summarizes activities to explore non-animal approaches for more complex endpoints, such as developmental toxicity and carcinogenicity testing, and resources to support development of 21st century testing approaches and computational toxicology capabilities. These include improvements to NICEATM's Integrated Chemical Environment, exploration of new approaches to validation, development of machine learning approaches for modeling toxicity endpoints and extraction of literature data, and analyses of variability of legacy in vivo data.

ICCVAM continues to expand outreach efforts to raise awareness of available alternative methods and foster partnerships with stakeholders. ICCVAM convened two Public Forum meetings, which generated productive interactions and allowed ICCVAM agency representatives to present updates on activities. Public meetings of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) focused on substantive strategic and technical discussions to leverage the SACATM members' expertise in advancing the ICCVAM mission. This report also provides summaries of two ICCVAM Communities of Practice webinars and over a dozen workshops, webinars, or webinar series

presented by ICCVAM agencies that focused on alternatives to animal testing. Many of these were organized in collaboration with nongovernment stakeholders, further strengthening relationships with these groups.

Many of the accomplishments described herein would not have been realized without the visionary leadership of Linda Birnbaum, Ph.D., who retired last year as director of the National Institute of Environmental Health Sciences. We thank her for the support and direction she provided to NICEATM and ICCVAM. We would also like to acknowledge the contributions of the representatives and interagency workgroup members from the 16 ICCVAM member agencies, as well as contributions of NICEATM and its contract staff at Integrated Laboratory Systems, the members of SACATM, and our many other stakeholders.

The activities summarized in this report exemplify ICCVAM's ongoing commitment to working with U.S. and international partners to advance development and acceptance of new scientifically valid test methods that will reduce and eventually replace animal use. We look forward to continued progress and effective interactions with our stakeholders in the coming years.

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Key NICEATM and ICCVAM Accomplishments and Impact

Key accomplishments of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and ICCVAM member agencies in support of the ICCVAM mission during 2018 and 2019 include:

- Publication of ICCVAM Strategic Roadmap. This document, developed with participation from the 16 ICCVAM member agencies, describes a framework for safety testing that will provide more human-relevant toxicology data while reducing the use of animals. To facilitate access to the roadmap by ICCVAM's international partners, the roadmap was translated into five languages.
- Development of the Collaborative Acute Toxicity Modeling Suite. The ICCVAM Acute Toxicity Workgroup organized a global project to develop in silico models of acute oral systemic toxicity that predict five specific endpoints needed by regulatory agencies. This project produced the Collaborative Acute Toxicity Modeling Suite, an online resource for screening organic chemicals for acute oral toxicity.
- Expansion of the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) Integrated Chemical Environment. NICEATM created the Integrated Chemical Environment to provide curated data and tools for safety assessment of chemicals. Updates of the Integrated Chemical Environment during 2018 and 2019 allowed users to run in vitro to in vivo extrapolation analyses, access quantitative structure-activity relationship predictions of endocrine activity and chemical properties, and explore data on mixtures.
- Department of Defense (DoD) strategic roadmap to promote the use of new approach methodologies in rapid chemical assessment. Currently in development, this DoD-specific roadmap will help laboratories across DoD to better define their chemical assessment needs and collaborate on development or refinement of appropriate non-animal approaches for testing.
- U.S. Environmental Protection Agency (EPA) draft policy on non-animal methods for skin sensitization. In 2018, EPA announced a draft science policy to reduce animal use in testing strategies that evaluate chemicals for their ability to cause an allergic

reaction, inflammation, or sensitization of the skin. The draft policy was the result of national and international collaboration.

- EPA initiatives to reduce animal use. In 2019, EPA announced a directive to prioritize efforts to reduce animal testing, which called for reducing mammal study requests and funding 30% by 2025 and completely eliminating them by 2035. EPA has also established criteria for waiving some types of animal study requirements.
- EPA strategic plan for the Toxic Substances Control Act. In 2018, EPA released a plan for that describes how the agency will promote development and implementation of methods and strategies that reduce, refine, or replace vertebrate animal testing to provide chemical safety information required under the Toxic Substances Control Act.
- U.S. Food and Drug Administration (FDA) predictive toxicity roadmap implementation. In 2017, FDA published its Predictive Toxicology Roadmap for integrating predictive toxicology methods into safety and risk assessments. FDA held meetings in 2018 and 2019 to solicit comments on the roadmap and highlight work done to support and implement it.

Technology

Many ICCVAM member agencies are developing new technologies and resources intended to reduce or replace the use of animals for chemical safety testing. These include platforms such as microphysiological systems (MPS) and data resources to support the development of predictive models and adverse outcome pathways (AOPs).

Assay Development

Many ICCVAM member agencies are developing new in vitro technologies and resources intended to reduce or replace animal use for chemical safety testing. They include technologies such as MPS and image analysis, and address important endpoints such as cardiotoxicity, inhalation toxicity, and neurotoxicity.

Prediction of differential responses to toxicity from genetically diverse cell lines

Tags: DoD

To gain a deeper understanding of the biological effects of chemical exposures, the U.S. Air Force Research Laboratory (AFRL) has developed a cell-based toxicity analysis system based on the Clarity Bioanalytics software platform. In the lab, cells are exposed to chemical or biological agents and imaged using high-content, high-resolution microscopy. These images are processed through an analytics pipeline using U.S. Department of Defense (DoD) supercomputing resources. The Clarity Bioanalytics system studies the changes in cells after exposure, identifies the level of toxicity of different compounds, and discovers genetic elements (e.g., single nucleotide polymorphisms, genes, pathways) that could affect the cellular risk to certain exposures. This software platform represents a central analytics tool for exposure toxicology research within AFRL. It allows unbiased phenotyping of the toxic effects of a variety of chemical and biological agents, and genotype-phenotype analyses for personalized response assessment and prediction. Initial experiments on this system were performed using B-lymphocytes in suspension, but ideally the system must be able to analyze images from a wide variety of cell types with the best possible accuracy. Current efforts have established machine learning methods to allow identification of any cell type and extract feature/phenotype information from cells, allowing expanded capabilities for comparative, unbiased phenotyping.

Gut-endothelial barrier models incorporating microbial colonies

Tags: DoD, MPS

AFRL has developed gut-endothelial barrier models using microfluidic technology. These gut-on-a-chip platforms emulate many of the microstructures (gut epithelial-blood endothelial interactions), morphologies (macro- and micro-villus structures), and functions of the human gut. The dynamic nature of the models provides a robust opportunity for assessing gut-blood barrier integrity, nutrient transport, and host-microbiome interactions. Moreover, a tailored oxygen environment, simulating the low-oxygen conditions seen in the human gut, promotes a more robust microbial colony inclusion. Current investigations are evaluating probiotics, cooperative microbial interactions, and complex microbial community dynamics in the gut-on-a-chip platform. The gut-on-a-chip models are also being combined with brain-on-a-chip models to allow analyses of complex molecular gut-brain axis interactions. These technologies in combination have the potential to enable assessment of operational stress and exposure outcomes due to thermal burden, nutrition, toxin ingestion and digestion, and their corresponding impact on the blood-brain barrier and neuronal activity. Furthermore, identification of molecular targets or pathways for countermeasure development can be developed, matured, and optimized in a human model prior to in vivo testing, thereby limiting animal usage and reducing the cost and time for deployment. Further validation and assessments of these promising capabilities will have to be completed before complete implementation.

Cell-based assays to identify chemical targets

Tags: DoD

The U.S. Army Combat Capabilities Development Command Chemical Biological Center (CCDC CBC) has been assessing and validating a number of cell-based in vitro platforms, which are currently being optimized as predictive toxicological tools. As a result of these efforts, the center has a fully functional, high-throughput G protein-coupled receptor screening assay, as well as single receptor tests using engineered cell lines to assay ion channel effects. These cell-based platforms should allow rapid assessment of the effects of different chemical threats.

Thus far, the center has used the PRESTO-TANGO assay system to test the activity of alpha adrenergic receptor agonists against an entire library of G protein-coupled receptors. Data currently collected align with results obtained using in vivo systems. Current and future efforts aim to test G protein-coupled receptor agonism and antagonism of a number of different compounds of interest.

Phenotypic screening platforms for cardiotoxicity, hepatotoxicity, and neurotoxicity

Tags: DoD, MPS, cardiotoxicity, hepatotoxicity, neurotoxicity, stem cells

CCDC CBC is developing, assessing, and implementing approaches that use MPS to evaluate complex phenotypes such as organ function. In this manner, three-dimensional, human cell-based platforms are being developed to better represent organ function and, more importantly, potential perturbation of normal phenotypes critical to health. These target organ effects are measurable on a phenotypic level, which serves as a mechanism to observe potential toxic effects without screening for a specific target of interest. Some examples of implementation efforts include:

- Cardiotoxicity can be measured in induced pluripotent stem cell (iPSC)-derived cardiomyocytes that synchronously beat in culture, with toxicity measured in real-time using impedance-based technology.
- Hepatoxicity can be measured using the Emulate Biotech liver chip system and the CN Bio PhysioMimix system, which have allowed success in the prolonged maintenance of a fully functional and viable liver environment in vitro. As a result, CCDC CBC can confidently assess both acute and chronic physiologically relevant, molecular-level hepatic effects of chemical threats without having to use an animal model.
- Neurotoxicity can be measured in two- or three-dimensional cultures of primary or iPSC-derived neurons that can be functionally assessed using microelectrode-based measurements of synaptic firing or cytotoxic endpoints measured via high-content fluorescent imaging.

Lung-on-a chip models to assess toxicity of aerosols

Tags: DoD, MPS, inhalation toxicity

AFRL has developed in vitro lung modeling tools, including lung-on-a-chip models, to enable robust toxicological assessments of aerosolized particulates. These models include technologies to aerosolize, characterize, and monitor dosimetry in real time. They also provide the control necessary to reproduce operational exposure conditions. The lung-on-a-chip models have cyclical stretch capability, simulating the mechanics of breathing, which has been shown to be important in the exposure mechanism. Together, these models and tools provide a platform to assess operationally relevant environmental or engineer toxicant exposures.

Human stem cell neuronal platform with phenotypic and physiological output

Tags: DoD, neurotoxicity, stem cells

The Biological Modeling Group of the AFRL 711th Human Performance Wing in collaboration with the Sanford Burnham Prebys Medical Discovery Institute is developing a human in vitro stem cell assay system to determine the mechanism of neurotoxins that potentially affect human brain function. This research includes benchmark testing of over 200 neurotoxins with known mechanisms. Effects on treated cells are assessed by neurophysiology (multi-electrode array), mitochondrial membrane potential changes, and reactive oxygen species generated in the cell culture system. The results of these ongoing studies are being analyzed to develop predictive toxicology capabilities using quantitative structure-activity relationship (QSAR) analysis and read-across computational methods. These approaches will enable predictions of toxicity of unknown chemicals with varying degrees of confidence, depending on how similar the unknown chemicals are to chemicals in the known database. The next phase of human neuron stem cell readout will involve a mechanistic large-scale neural network modeling of chemically induced changes in the in vitro firing activity of the networking neurons. By measuring these changes in networking firing rates in combination with behavior phenotypes in the case of known chemical entities, the goal is to be able to predict the response for unknown chemicals or chemicals with limited experimental data.

Human Cancer Models Initiative

Tags: NCI, carcinogenicity

The Human Cancer Models Initiative is an international consortium that is generating novel, next-generation, tumor-derived culture models annotated with genomic and clinical data. Models and related data developed through the initiative are available as a community resource. The National Cancer Institute (NCI) is contributing to the initiative by supporting four Cancer Model Development Centers. These centers are tasked with producing next-generation cancer models, such as organoids and conditionally reprogrammed cells, from clinical samples. The cancer models include tumor types that are rare, originate from patients from underrepresented populations, lack precision therapy, or lack cancer model tools. In 2018, a funding opportunity was offered to increase the racial and ethnic diversity of the samples used to develop new culture models.

Cancer Tissue Engineering Collaborative

Tags: NCI, carcinogenicity

The NCI Cancer Tissue Engineering Collaborative Research Program supports the development and characterization of state-of-the-art biomimetic tissue-engineered technologies for cancer research. Collaborative, multidisciplinary projects that engage the fields of regenerative medicine, tissue engineering, biomaterials, and bioengineering with cancer biology will be essential for generating novel experimental models that mimic cancer pathophysiology to elucidate specific cancer phenomena that are otherwise difficult to examine in vivo. Projects are funded through grants offered by NCI; the current round of funding opened in 2019 and will continue through January 2022. Endpoints under investigation by current projects include brain, breast, ovarian, and colon cancer.

Biomimetic in vitro models for assessment of occupational respirable aerosol hazards

Tags: NIOSH, inhalation toxicity

Inhalation of respirable particles in the workplace can cause lung disease. Risk assessment of respirable particles exposure has been challenging because of the large number of new particulate chemicals used in the workplace and the limited availability of appropriate in vitro models for toxicity assessment of respirable particles. To address this challenge, scientists at the National Institute for Occupational Safety and Health developed in vitro cell culture models that allow simple, rapid, and specific testing of respirable particle toxicity as well as detailed mechanistic investigations of exposure effects. The models employ human

lung cells for potential use in the screening of occupational respiratory hazards and for potential use in risk assessment. Considerations include determining the relevant in vitro doses to reflect potential occupational exposures, specific lung cell types, and disease endpoints. In vitro models are being used to assess both acute toxicity hazard and cancer hazard from long term exposure to low doses of particles (Kornberg et al. 2019). Further improvements to these in vitro models are being made by integrating the 3D liquid-air interface platform to simulate the complexity of real respirable particle airway exposure conditions. Successful development of this integrated system will facilitate rapid and realistic assessment of respirable particle toxicities.

Computational Tools Development

Computationally generated predictions of toxicity endpoints can inform decisions about testing priorities and sometimes eliminate the need for laboratory testing. ICCVAM agencies are developing tools to predict toxicity endpoints such as acute oral toxicity, inhalation toxicity, and endocrine disruption, as well as tools to relate in vitro data to in vivo toxicities through in vitro to in vivo extrapolation (IVIVE).

Collaborative Modeling Project for Androgen Receptor Activity (CoMPARA)

Tags: EPA, NIEHS, endocrine disruptors

EPA established the worldwide Collaborative Modeling Project for Androgen Receptor Activity (CoMPARA) consortium to develop computational approaches to screen chemicals for their potential androgenic activities. CoMPARA followed the approach used for the Collaborative Estrogen Receptor Activity Prediction Project (CERAPP) (Mansouri et al. 2016).

Regulatory requirements to screen chemicals for potential endocrine disrupting activity are being addressed via high-throughput screening (HTS) approaches and computational modeling. CoMPARA (Mansouri et al. 2020) brought together scientists from 25 international groups, who contributed 91 predictive QSAR models to predict androgen receptor binding, agonist, and antagonist activity. Models were evaluated using literature data extracted from different sources and curated for quality. To overcome the limitations of single-model approaches, CoMPARA predictions were combined into consensus models that provided averaged predictive accuracy of approximately 80% for the evaluation set. These consensus models have been implemented into the free and open-source Open Structure-activity/property Relationship App (OPERA) application to enable new chemicals of

interest to be screened. The entire EPA DSSTox (Distributed Structure-Searchable Toxicity) database of ~750,000 chemicals was virtually screened. Predicted androgen receptor activities have been made available on the EPA CompTox Chemicals dashboard and in the Integrated Chemical Environment (ICE).

Collaborative Acute Toxicity Modeling Suite (CATMoS) tool for predicting acute oral toxicity

Tags: ICCVAM, NIEHS, acute toxicity, oral toxicity

The Collaborative Acute Toxicity Modeling Suite (CATMoS) is a free online resource for screening organic chemicals for acute oral toxicity.

The ICCVAM Acute Toxicity Workgroup organized a global project to develop in silico models of acute oral systemic toxicity that predict five specific endpoints needed by regulatory agencies:

- Very toxic (LD50 <50 mg/kg vs. all others)
- Nontoxic (LD50 >2000 mg/kg vs. all others)
- LD50 point estimates
- Hazard categories under the U.S. Environmental Protection Agency (EPA) classification system (n=4)
- Hazard categories under the United Nations Globally Harmonized System for Classification and Labelling of Chemicals (GHS: n=5; Category 5 and Not Classified combined into a single category)

The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) invited scientists to develop models to predict any or all of these endpoints. NICEATM and the EPA National Center for Computational Toxicology (NCCT; now part of the EPA Center for Computational Toxicology and Exposure) collected a large body of rat acute oral toxicity data. Subsets of these data were used by project participants to build and test their models, and by NICEATM and the project organizing committee to evaluate the models. Project participants and potential users of the models attended a workshop at the National Institutes of Health in Bethesda, Maryland, in April 2018 (Kleinstreuer et al. 2018).

Models developed for the project that met both quantitative and qualitative criteria defined by the project organizing committee were used to generate consensus predictions for the acute oral toxicity endpoints of interest to regulatory agencies. The consensus predictions are available in CATMoS, which is implemented in v2.5 of OPERA, a free and open-source QSAR tool (Mansouri et al. 2018). The consensus predictions will also be available via ICE and EPA's CompTox Dashboard. A journal article in preparation for submission in 2020 will describe generation of the consensus predictions.

Open Structure-activity/property Relationship App (OPERA)

Tags: EPA, NIEHS

OPERA is a free and open-source/open-data suite of QSAR models providing predictions for physicochemical properties, environmental fate parameters, and toxicity endpoints. OPERA is an ongoing collaboration between NICEATM and EPA.

QSAR models provide predictions of chemical activity that can augment non-animal approaches for predicting toxicity. However, the performance of QSAR models highly depends on the quality of the data and modeling methodologies used. EPA NCCT created OPERA to provide robust QSAR models for chemical properties of environmental interest that can be used for regulatory purposes (Mansouri et al. 2018).

Additions to OPERA during 2018-2019 include predictions for:

- Estrogenic activity from CERAPP (Mansouri et al. 2016).
- Androgenic activity from CoMPARA (Mansouri et al. 2020).
- Acute oral systemic toxicity from CATMoS (Kleinstreuer et al. 2018).
- Physicochemical properties such as acid dissociation constant (pKa) and octanol-water dissociation coefficient (logD) (Mansouri et al. 2019).
- Properties such as human plasma fraction unbound and hepatic intrinsic clearance that affect how substances behave in biological systems, to support an open-source workflow for IVIVE.

ICE tools for IVIVE and chemical characterization

Tags: NIEHS, IVIVE

NICEATM created ICE to provide curated data and tools to facilitate the safety assessment of chemicals. Launched in 2017, ICE addresses the data needs frequently expressed by NICEATM stakeholders. The May 2019 ICE 2.0 update provided a new user interface to simplify searches and new tools that let users explore chemical properties and toxicity in more detail. Tools provided in the update included:

- An update of the IVIVE tool (Casey et al. 2018) to provide more complex models, including those from EPA's high-throughput toxicokinetic (httk) package, that can improve prediction accuracy.
- Chemical list characterization to explore physicochemical properties of chemical sets.

High-throughput in vitro to in vivo extrapolation using Tox21 data

Tags: NIEHS, Tox21, IVIVE

This National Institute of Environmental Health Sciences (NIEHS) project developed and refined approaches to extrapolate all Tox21 chemical-concentration effect data to estimate human-equivalent exposure doses. The effort built on previous efforts using high-throughput toxicokinetics models and combined them with in silico-estimated parameters. A publicly available web application based on these methods is available through the Tox21 Toolbox on the NTP public website. NIEHS is continuing to refine the models for use in research and chemical screening prioritization. Additionally, a simple IVIVE workflow allowing users to select Tox21 assays and chemicals and extrapolate to estimated exposures is available in ICE. The ICE IVIVE tool provides three models with varied complexity and exposure routes: a one-compartment pharmacokinetic (PK) model, a three-compartment PK model, and a multi-compartment physiologically based PK (PBPK) model for oral and intravenous routes. These models use the ICE curated Tox21 data to predict equivalent administered in vivo doses for acute oral toxicity and endocrine disruption endpoints.

Optimization of pharmacokinetic models for in vitro to in vivo extrapolation of estrogenic activity

Tags: NIEHS, IVIVE, endocrine disruptors

NIEHS scientists evaluated and optimized IVIVE approaches using in vitro estrogen receptor activity to predict estrogenic effects measured in rodent uterotrophic studies. This work

(Casey et al. 2018) evaluated the use of three PK models with varying complexities to extrapolate in vitro to in vivo dosimetry for 29 estrogen receptor agonists using data from validated in vitro and in vivo methods. In vitro activity values were adjusted using mass-balance equations to estimate intracellular exposure via an enrichment factor, and steady-state model calculations were adjusted using fraction of unbound chemical in the plasma to approximate bioavailability. Accuracy of each model-adjustment combination was assessed by comparing model predictions with lowest effect levels from guideline uterotrophic studies. The comparison found little difference in model predictive performance based on complexity or route-specific modifications. Simple adjustments, such as using the enrichment factor to account for in vitro intracellular exposure or fraction of unbound chemical to account for chemical bioavailability, resulted in significant improvements in the predictive performance of all models. The resulting computational IVIVE approaches accurately estimated chemical exposure levels that elicit positive responses in the rodent uterotrophic bioassay. Such studies are important for establishing confidence in the quantitative extrapolation of in vitro activity to relevant end points in animals or humans.

Semi-automated extraction of literature data using machine learning methods

Tags: NIEHS, DOE

Identifying and extracting information from the full text of scientific publications is a critical step required in developing reference databases for establishing confidence in alternative approaches. However, manually extracting protocol details such as species, route of administration, and dosing regimen is labor-intensive and can introduce errors. NIEHS and the Department of Energy's Oak Ridge National Laboratory are applying natural language processing and machine learning methods using both unsupervised and supervised approaches to identify specific data elements in the full text of scientific publications. For example, an unsupervised approach was developed to identify text segments (sentences) relevant to a set of criteria describing specific study parameters, such as species, route of administration, and dosing regimen. A binary classifier was then trained to identify publications that met the criteria. The classifier performed better when trained on the candidate sentences than when trained on sentences randomly picked from the text, supporting the hypothesis that this method could accurately identify study descriptors. This

work is being expanded to include machine learning-based multivariate models combined with natural language processing to automatically extract text features that correspond to study descriptors and classify papers based on their adherence to minimum criteria derived from regulatory guideline studies. A publication is being drafted for submission in 2020.

Pred-Skin web portal for predicting human skin sensitizers

Tags: NIEHS, skin sensitization

In collaboration with scientists at the University of North Carolina at Chapel Hill and the Universidade Federal de Goiás, NICEATM scientists contributed to the development of a model to predict skin sensitization potential of chemicals for two assays, human patch test and murine local lymph node assay, and implemented this model in a web portal (Braga et al. 2017). Work over the last two years focused on substantially revising and expanding the freely available web tool, Pred-Skin version 3.0, to integrate multiple QSAR models developed with in vitro, animal in vivo, and human ex vivo data into a consensus naïve Bayes model that predicts human effects. All models are freely accessible through the Pred-Skin v. 3.0 portal. A publication is being drafted for submission in 2020.

AoPXplorer

Tags: DoD, adverse outcome pathways

AOPXplorer is a tool to visualize non-animal assay data, mRNA levels, protein expression, and molecular biology data within an AOP network. The networks in AOPXplorer are built by U.S. Army scientists and the user community and are typically based on disease pathways and known modes of action for chemicals. During 2018 and 2019, several new pathways in AOPXplorer were developed and updated, including steatosis, signal transduction, regenerative proliferation, learning memory and cognitive decline, general cancer pathway, estrogen-mediated breast cancer pathway, PPAR-gamma lung fibrosis pathway, steroidogenesis, and skin sensitization. As of January 2020, AOPXplorer has been downloaded over 950 times. AOPXplorer is an app for the Cytoscape network visualization software and can be downloaded from the Cytoscape app store. Submissions of new pathways to AOPXplorer are welcomed.

Computational neuronal network analysis for extrapolation of iPSC findings to human in vivo effects

Tags: DoD, neurotoxicity, stem cells

AFRL is developing an in silico mechanistic model of an in vitro experimental system consisting of human iPSC-derived dopaminergic neurons cultured on microelectrode arrays. While microelectrode array recordings of cultured neurons are useful for large, high-throughput experiments, cultured neurons form synaptic connections randomly rather than functionally as in the central nervous system, making it difficult to compare the firing activity recorded via microelectrode array to activity patterns produced by the human brain. This model should make it possible to extrapolate in vitro results to an expected impact on brain functions such as cognition and memory. An initial version of the model was implemented in the NEST modeling software. This combined platform contained neuronal types similar to those found in the human iPSC-derived neuronal cultures, parameterized using human and rodent data from the literature. Some key aspects of the behavior of the human iPSC-derived neurons could not be accurately represented due to limitations in the NEST software; the model was able to reproduce biological firing patterns qualitatively, but with unrealistically fast kinetics. The model is currently being redeveloped in the NEURON modeling software, which allows a more detailed implementation of neuronal mechanisms. This will allow the key behaviors of the human iPSC-derived neurons to be represented accurately.

Machine learning approaches to assess new threat compounds

Tags: DoD, acute toxicity

CCDC CBC is actively investigating machine learning approaches to assess new threat compounds. Specifically, the center is focused on three major initiatives focused on characterizing the toxicity of emerging threat compounds. These include:

- Regression/classification models for assessing acute toxicity in non-human species.
- Prediction of chemical targets using available in vitro data on human cell systems.
- Prediction of chemical-target interaction with three-dimensional docking models.

Generalized inhalation toxicity model

Tags: EPA, DoD, inhalation toxicity, acute toxicity

The AFRL 711th Human Performance Wing is collaborating with EPA to create an inhalation component for the R-based httk package. The goal of this effort is to develop a computational approach to generate high-throughput toxicokinetic estimates of chemical inhalation exposures. The inhalation component of httk will have three major modules: gas, aerosol, and mixture inhalation. In combination, these modules will address the most common occupational exposure scenarios. The project utilizes open-source software, which should encourage ongoing collaborations for updating and improving the models.

The gas inhalation module utilizes physicochemical property values from the OPERA QSAR tool to estimate the time course of blood and exhaled breath concentrations. A paper describing the gas inhalation module in more detail will be published in 2020 (Linakis et al. 2020). The aerosol module currently includes updated versions of two previously published lung deposition models to allow users more flexibility in determining which region of the lungs may be affected as a result of exposures to aerosols of a certain particle size. Upon completion and release of these modules, a mixture module will be pursued utilizing the current literature on gas-aerosol/particle interactions.

Expanded Decision Tree software for toxicity classification

Tags: FDA

FDA is developing the Expanded Decision Tree software, which will be a free tool to classify compounds into six classes of relative toxic potential.

During the last seven decades, scientific advancements have led to an exponential increase in the number and types of chemicals to which humans are known to be exposed, leading to an ever-increasing need to screen and prioritize these substances according to their relative toxicity. The Cramer et al. (1978) Decision Tree is a screening and prioritization tool that sorts chemicals into three classes of relative toxicity. FDA updated and expanded the Cramer et al. Decision Tree to reflect the current state of the science and to make the decision tree applicable to a broader scope of substances, including those present in food, food contact materials, cosmetics, and dietary supplements. Additionally, FDA increased the number of

classes of relative toxicity to six (non-toxic, low, medium, high, very high, and extreme toxicity) and quantified the toxic potential of each class by calculating a threshold of toxicological concern level for each class. By screening and prioritizing substances, the Expanded Decision Tree software will help focus resources on the safety assessments of substances with greater potential for public health risk and help reduce the use of animals for safety testing. An update on the development of the Expanded Decision Tree was presented at the September 2019 FDA workshop, "Implementing FDA's Predictive Toxicology Roadmap: An Update of FDA Activities," which highlighted activities to support and implement its Predictive Toxicology Roadmap.

Data Resources

As momentum grows toward adoption of alternative methods for chemical safety testing, curated data are needed to support method validation and establish scientific confidence in new approaches. ICCVAM agencies address that need by compiling data and making them publicly available.

Integrated Chemical Environment updates

Tags: NIEHS, Tox21

NICEATM's ICE provides data and tools to help develop, assess, and interpret chemical safety tests. An update of ICE was launched in May 2019 that provides a home page with links to all ICE tools and detailed user guides. An update of the Search tool simplified access to chemical information, and tools were added for IVIVE and chemical characterization.

Other data updates to ICE during 2018 and 2019:

- Expanded physicochemical property predictions to include over 720,000 chemicals using an updated set of predictive models (Mansouri et al. 2018).
- Updated the curated HTS data coming from the Tox21 initiative to include increased curation and the most up-to-date data release.
- Added predictions of endocrine activity using the CERAPP (Mansouri et al. 2016) and CoMPARA (Mansouri et al. 2020) models.

Unsupervised data-driven analysis of Tox21 assay data

Tags: NIEHS, Tox21

Scientists at NIEHS are using computational data organization methods based on patterns to identify chemicals that exhibit biological properties similar to those of well-characterized toxicants in the Tox21 10K library. Users will soon be able to perform bioactivity-based correlations to compare query chemicals with well-characterized Tox21 toxicants using the Tox21 Correlation Browser. An upgrade to the Correlation Browser is in progress and an improved web tool will be available in 2020.

Results of these analyses are being used in combination with another tool, Tox21 Enricher, to help prioritize compounds for more extensive toxicological testing. The Tox21 Enricher performs neighborhood enrichment analysis to determine if one or more input chemicals have characteristics that show similar bioactivity patterns to characterized chemicals. Tox21 Enricher has been described in a publication (Hur et al. 2018).

Tox21 high-throughput screening assay target mapping

Tags: NIEHS, Tox21

NICEATM is mapping assay targets used in Tox21 and the EPA Toxicity Forecaster (ToxCast) programs to known modes of action for developmental toxicity, acute toxicity, and carcinogenicity based on established modes of action from literature. The assay mappings are used in ICE to facilitate targeted access to the Tox21 data in ICE tools. This mapping draws on established ontologies and controlled vocabularies such as the NCI Metathesaurus and UMLS Metathesaurus to promote the interoperability of ICE data with partners.

Compilation of human skin sensitization data

Tags: CPSC, FDA, NIEHS, skin sensitization, IATA

To support the evaluation of non-animal approaches for skin sensitization assessment, scientists from the U.S. Consumer Product Safety Commission (CPSC), the U.S. Food and Drug Administration (FDA), and NICEATM worked with industry and international collaborators to collect human predictive patch test data from approximately 1800 publications. Results considered to be sufficiently reliable were classified using GHS categories. Decision tree and weight-of-evidence approaches were used to help resolve

ambiguity and discordance in individual tests for each substance. This classification approach was applied to a Cosmetics Europe reference list of 128 substances to support the evaluation of defined approaches for skin sensitization proposed for inclusion in a new Organisation for Economic Co-operation and Development (OECD) guideline, for which reliable classifications were obtained for 80 substances. Results of the analysis were described in an abstract (Strickland et al.) accepted for presentation at the 2020 Society of Toxicology Annual Meeting. The entire human skin sensitization patch test database will be made publicly available in the future for additional evaluation of alternative skin sensitization methods and development of new models.

Extraction and annotation of legacy developmental toxicity study data

Tags: NIEHS, developmental toxicity

To support the evaluation of non-animal approaches for developmental toxicity assessment, NICEATM scientists extracted information from about 250 NTP legacy prenatal developmental toxicity animal studies and a subset of about 50 studies submitted to the European Chemicals Agency (ECHA) that were deemed high-quality by NTP subject matter experts. Study details extracted included species, strain, administration route, dosing duration, and treatment related effects. Efforts are underway to standardize the effects extractions by applying controlled vocabularies and ontologies to facilitate computational analyses and integration with other structured databases such as EPA's ToxRefDB.

Variability analysis of in vivo data to set performance thresholds for alternative methods

Tags: NIEHS, acute toxicity, oral toxicity, skin irritation

To better characterize the reproducibility of the in vivo rabbit skin irritation assay, NICEATM assessed variability within a data set of over 3000 results for over 700 chemicals tested at least twice. The in vivo rabbit skin irritation assay has historically been the benchmark against which new approach methodologies (NAMs) have been compared. However, a limiting factor in identifying a full replacement for the in vivo method could be the variability inherent to the subjective scoring of responses in the rabbit test. The NICEATM analysis found that chemicals classified as moderate irritants at least once were classified as mild irritants or non-irritants over 40% of the time when tested repeatedly. The level of variability was greatest between mild and moderate irritant classifications. This

analysis indicates that the level of variability present in the rabbit skin irritation test should be taken into consideration when evaluating the performance of non-animal alternative methods.

NICEATM also assessed the reproducibility of the rat acute oral lethality test using a comprehensively curated dataset of over 2000 chemicals. Quantitative and categorical analyses were conducted and a global confidence interval was established to characterize the inherent variability of the test method. These analyses revealed that independent studies can yield LD50 values orders of magnitude apart, which can have significant implications from a regulatory perspective due to the resulting impact on hazard classification and labeling. Similar to the analysis of the rabbit skin irritation test, no physicochemical properties or differences in test method protocol were identified that could readily explain differences between study results. The resulting publicly available dataset can be used for modeling, and the calculated confidence interval can be applied when assessing performance of NAMs. A publication describing this work is planned for submission in 2020.

Establishment of ontologies for zebrafish developmental toxicity screening studies
Tags: NIEHS, developmental toxicity

NICEATM is coordinating collaborative projects to establish ontologies for zebrafish screening. Three laboratories tested the same 90-chemical set using similar study designs and morphology assessments. Data from these studies are being curated and assessed to refine testing approaches and support development of informatics resources. A poster describing this project (Ceger et al.) was presented at the 2019 annual meeting of the Society for Birth Defects Research and Prevention (formerly the Teratology Society).

To further define ontologies for zebrafish screening, NICEATM coordinated an online evaluation of heterogeneity in terms used to describe zebrafish phenotypes following chemical exposure. Zebrafish images were posted online for researchers to evaluate the phenotypes using their in-lab terminology. Results were collected, compiled, and terms mapped to the Zebrafish Phenotype Ontology. A second online evaluation is scheduled for early 2020 and will employ controlled vocabulary; results of this evaluation will be presented in a NICEATM webinar and published.

Retrospective analysis of acute fish toxicity data

Tags: EPA, NIEHS, acute toxicity, ecotoxicity

To assess potential hazards to wild fish species, EPA currently requires testing in each of three different fish types: warmwater, coldwater, and marine/estuarine. NICEATM and the EPA Office of Pollution Prevention and Toxics are extracting and evaluating acute fish toxicity data from pesticide safety studies submitted to the EPA office. These data will be analyzed to determine whether one or more of the three fish types can be eliminated from testing requirements. Extracted data will be submitted to EPA's Toxicological Reference Database.

Retrospective analysis of triple-pack studies for dermal absorption

Tags: EPA, NIEHS

To provide an estimate of dermal absorption, the EPA Office of Pesticide Programs combines data from in vivo rat, in vitro rat, and in vitro human dermal absorption studies, commonly referred to as the "triple pack", to calculate a chemical-specific dermal absorption factor. The dermal absorption factor estimates the percentage of the external dose that will cross the skin barrier and end up in the systemic circulation. To assess the feasibility of using data from in vitro studies alone to estimate this value, EPA and NICEATM, in conjunction with the Health Canada Pest Management Regulatory Agency and industry stakeholders, are conducting a retrospective analysis of triple pack reports for agrochemicals completed between 2005 and 2015. Data were extracted from study reports provided to NICEATM by EPA and agrochemical products companies ADAMA, BASF, Bayer, Corteva, FMC, Syngenta, and Valent. The aim of this analysis is to determine whether the rate of in vitro absorption is equal to or greater than the rate of in vivo absorption. Absorption values from in vitro rat studies are also being compared to in vitro human studies to determine the differences in permeability between rat skin and human skin. A stakeholder meeting was convened in May 2019 at EPA to discuss the preliminary findings from this review and to identify and discuss additional information needs for both U.S. and Canadian regulators to finalize the assessment. A paper detailing this work is planned for submission in 2020.

Human BioMolecular Atlas Program

Tags: NIH

In October 2018, the National Institutes of Health (NIH) issued its first set of research funding awards for the Human BioMolecular Atlas Program. This is an open, global framework to support the research community's efforts to map the adult human body at the level of individual cells. The project will award $54 million between 2018 and 2021 to support:

- Generating, standardizing, and validating data sets on cell organization and variability.
- Developing new tools and techniques to construct high-resolution tissue maps.
- Coordinating program activities, managing Human BioMolecular Atlas Program data, and building an atlas of tissue maps.

Changes to NLM TOXNET resources

Tags: NLM

The National Library of Medicine (NLM) created the Toxicology Data Network (TOXNET) in 1985 to improve access to NLM's online chemical and toxicity databases. As part of the reorganization associated with its current strategic plan, NLM retired TOXNET in December 2019. Most of TOXNET's databases have been incorporated into other existing NLM resources. Information about how to access these databases is available on the NLM website, and more detailed information is available in an NLM Technical Bulletin issued in November 2019. New locations for other NLM resources such as ToxTutor and ALTBIB are under discussion. NLM will conduct webinars and other presentations in 2020 to inform former TOXNET users how they can continue to access these resources.

Tox21 Cross-partner Projects

Tox21 is a collaboration among groups within four U.S. federal organizations aimed at developing more efficient approaches to predict how chemicals may affect human health. Tox21 studies use assays that are run at higher throughput than traditional tests. The goal of Tox21 is to use data from these assays to prioritize substances for further evaluation, inform understanding of mechanisms of action, and/or develop improved predictive models for toxicity. Test approaches developed and data collected via this initiative may enable agencies

to reduce reliance on animal data for assessing chemical safety. Tox21 projects and projects using Tox21 data are described below and throughout this report.

The four groups participating in the Tox21 collaboration are ICCVAM members:

- U.S. Environmental Protection Agency
- U.S. Food and Drug Administration
- National Toxicology Program (within NIEHS)
- National Center for Advancing Translational Sciences (NCATS; of the National Institutes of Health)

Cell line selection for high-throughput transcriptomics

Tags: EPA, NIEHS, Tox21

Transcriptomics uses a cell's overall gene expression to assess many aspects of biology in the cell, including its normal function and response to toxicity. A question of interest is whether the cell types used in high-throughput transcriptomics assays need to reflect human biological diversity to identify different classes of toxicants and clarify the relevant biology for toxicity testing. To evaluate this question, EPA and NIEHS collaboratively used transcriptomics databases and other resources to identify cell lines that maximized biological diversity at the level of gene expression. Using a newer high-throughput transcriptomics technology, gene expression will be assessed in these cells under normal conditions and with chemical treatment. Ultimately, comparisons will identify selected cells for high-throughput transcriptomics chemical screening and also enlighten how future cell lines should be chosen. An abstract providing an update was accepted for presentation at the April 2020 meeting of the Midsouth Computational Biology and Bioinformatics Society.

In vitro disposition of Tox21 chemicals

Tags: EPA, NIEHS, IVIVE, Tox21

IVIVE relates chemical concentrations that induce a response in an in vitro assay to chemical exposures that induce relevant effects in vivo. IVIVE typically assumes that chemicals behave in cells in an in vitro system in the same way they behave in blood and tissue in animals. Significant differences between in vitro models and in vivo systems make this assumption inaccurate. While the nature and extent of these differences are not well

characterized, it is known that a chemical's physicochemical properties affect factors such as binding to plastic and partitioning between medium and cells. EPA and NIEHS scientists selected a group of chemicals from the Tox21 library representing a diversity of structural and physicochemical properties and are examining their in vitro disposition to better understand these factors. Work since 2018 has focused on a pilot set of 10-20 chemicals, with anticipated expansion to 100-200 chemicals in 2020. This anticipated work will represent the largest undertaking of empirical measures of in vitro disposition and inform model predictions of in vitro disposition for other chemicals beyond those tested.

High-throughput transcriptomic profiling of a diverse subset of Tox21 10K library chemicals

Tags: EPA, NIEHS, NIH, Tox21

High-throughput transcriptomics generates gene expression profiles to rapidly evaluate the effects of large numbers of chemicals on in vitro cell culture systems (Harrill et al. 2019). To provide a basis for characterizing the toxicity potential of chemicals with limited or no available data, scientists at NIEHS and EPA are building a common reference chemical dataset to enhance interpretation of high-throughput transcriptomics screening data. The project systematically identified a robust set of reference chemicals with direct interactions to specific biological targets (e.g., nuclear receptors, enzymes, kinases, ion channels). A subset of approximately 300 of these reference chemicals has been acquired by Tox21 chemistry collaborators for evaluation in two human cell culture models: MCF-7 cells, derived from breast cells, and HepaRG, derived from liver cells (Ramaiahgari et al. 2019). The next stage of the project is to create the reference chemical dataset, analyze both gene-level and pathway-level responses that enable improved interpretation of transcriptomic data with test chemicals, and identify the most efficient conditions to expand coverage to thousands of reference chemicals.

Predictive modeling of developmental toxicity with human pluripotent stem cells

Tags: EPA, FDA, NIEHS, developmental toxicity, IVIVE, Tox21

FDA and NICEATM scientists applied IVIVE to evaluate the impact of pharmacokinetics and different modeling approaches on predicting relevant external exposure from in vitro developmental toxicity potential concentrations derived from an in vitro human iPSC-based

assay. Previous work showed that the devTOX quick Predict assay ranked the developmental toxicity potential of valproate analogues in a manner that was consistent with observed developmental toxicity potency in vivo. The IVIVE analysis in this project estimated equivalent administered doses that would result in maternal blood concentrations equivalent to the developmental toxicity potential and cytotoxic in vitro concentrations. The estimated equivalent administered doses were compared to published lowest effect levels from in vivo developmental toxicity studies. Preliminary results of this analysis showed close agreement between equivalent administered doses and in vivo rat lowest effect levels for two valproate analogues. This suggested that the devTOX quick Predict assay and IVIVE approaches can quantitatively predict in vivo developmental toxicity potential. An abstract describing this work (Chang et al.) was accepted for a presentation at the Society of Toxicology (SOT) 2020 annual meeting.

Incorporating genetic susceptibility into developmental neurotoxicity screening via population diversity

Tags: EPA, FDA, NIEHS, developmental toxicity, neurotoxicity, Tox21

The potential for neurotoxicity in children following exposure to environmental chemicals remains a high public priority due to concerns about recent increases in the prevalence of neurological disorders such as attention deficit hyperactivity disorder and autism. Neurotoxicity risk for an individual depends on a number of factors, including interactions between an individual's variation in genetic makeup and exposures to neurotoxic chemicals in the environment. To investigate the role of genetic diversity in susceptibility to neurotoxicity, scientists at NIEHS, EPA, and FDA are using a genetically diverse set of cells to evaluate a curated set of chemicals with neurotoxic potential. Neural progenitor cells were derived from a set of mice bred to maximize genetic diversity, yielding 200 male and female genetically different cell lines. The panel of cell lines will be exposed to varying concentrations of the chemical test set and assessed using a high-content imaging assay called cell painting. The compiled dataset will be used to identify chemicals with a range of developmental neurotoxicity potencies. These data will inform data-driven uncertainty factors that account for interindividual variability, allowing for adequate protection of genetically sensitive subpopulations.

Performance-based validation of Tox21 assays

Tags: EPA, NIEHS, Tox21

To use data generated by HTS initiatives such as Tox21 and the EPA ToxCast program in regulatory applications, the assays and models built from the assays must be validated based on their performance against the biological targets they query. This requires developing sets of reference chemicals that consistently yield reproducible results when assayed for these biological targets. A process also needs to be established for assessing and reporting the performance evaluation of an assay in a standardized format that provides consumers of the data the ability to interpret the appropriate context for use of the assay. Finally, both the development of reference chemical sets and the validation process need to be streamlined and rapid enough to manage the tens to hundreds of assays that can help inform regulatory toxicity endpoints. To address these needs, scientists at EPA and NIEHS developed a process to identify reference chemicals that consistently produce positive or negative results when assayed in defined assays (Judson et al 2019). Additional work under this project has focused on identifying chemicals that consistently produce false signals by interfering with specific technology types and backgrounds. Models to predict interferent chemicals for luciferase inhibition and autofluorescence are available on the NTP website. Current efforts are focused on using these data to identify reference chemicals and establish protocols for evaluating the performance of specific Tox21 and ToxCast assays.

Retrofitting existing Tox21 HTS assays with metabolic capability

Tags: EPA, NIEHS, NIH, Tox21

The HTS assays that have been run in the Tox21 testing program to date generally lack the metabolic activity found in living systems, which can potentially increase or decrease the toxicity of chemicals. As a result, HTS results may not accurately reflect in vivo activity. Scientists at EPA, NCATS, and NIEHS are using several approaches to address this problem: adding human or rat liver microsomes into the existing assays, transfecting cells with mRNAs encoding human metabolic enzymes, or using metabolically capable human HepaRG cells. The addition of metabolic capacity to HTS assays is expected to improve characterization of the in vivo activity of chemicals in the Tox21 collection. Current efforts focus on retrofitting three types of assays for which a massive amount of data have already

been generated: cellular stress-related assays, endocrine disruption assays, and CYP450 enzyme inhibition assays. The retrofitted assays are being used to screen the Tox21 10K chemical library to identify chemicals that are either bioactivated or detoxified by metabolic activity.

Expansion of pathway coverage by Tox21 HTS assays for better prediction of adverse drug effects

Tags: NIEHS, FDA, NIH, Tox21, hepatotoxicity, cardiotoxicity, AOP

To date, Tox21 HTS assays have focused primarily on selected nuclear receptor and stress response pathways. This relatively limited focus suggests that activity in other toxicity pathways has not been adequately assessed; it is likely that some unexplored pathways relate to unanticipated adverse drug effects. Therefore, expanding the coverage of biological responses by adding assays that probe under-represented pathways in the current Tox21 assay portfolio may improve the predictivity of Tox21 data. Scientists at FDA, NIEHS, and NCATS are systematically identifying these under-represented pathways in a data-driven approach and nominating assays for development and Tox21 chemical screening. The data generated (Huang et al. 2018) will be used to build models for human toxicity prediction, focusing on common adverse drug effects such as drug-induced liver injury and cardiotoxicity. An initial panel of targets and pathways has been identified (Huang et al. 2019) using existing drug-target annotations and adverse effect information obtained from the literature and public databases, such as DrugBank. In parallel, human toxicity data are being collected and curated from the literature. These data will be used to target additional cellular pathways for future assay development and validation.

Profiling activity of acetylcholinesterase inhibitors

Tags: FDA, NIH, neurotoxicity, Tox21

Acetylcholinesterase inhibitors cause a variety of adverse effects in the nervous system. Some acetylcholinesterase inhibitors serve as drugs, while others are used as pesticides or found in natural products. Scientists at FDA and NCATS developed acetylcholinesterase inhibition assays (Li et al. 2017) and screened the Tox21 10K chemical library to identify environmental and drug- or food-related chemicals that inhibit acetylcholinesterase activity. The screening study also provided an opportunity to evaluate performance characteristics of

HTS assays intended to measure acetylcholinesterase inhibition. An accomplishment of the study included incorporating metabolism into the assays (Li et al. 2019), an important consideration as some acetylcholinesterase inhibitors become more potent when metabolized. Selected inhibitors were further characterized using stem cells and computational models to gain insights on their inhibitory mechanisms.

Confidence

Establishing confidence in new testing methods requires engagement by both federal agencies and stakeholders. To support this goal, ICCVAM agencies evaluate the suitability of new methods for specific purposes and facilitate communication and education about new methods.

Assay Application

ICCVAM and its member agencies conduct, support, and evaluate studies investigating the suitability of new laboratory methods for specific purposes. Methods evaluated during 2018 and 2019 addressed endpoints including inhalation toxicity, skin sensitization, eye irritation, and endocrine disruption.

Electrophilic allergen screening assay validation study

Tags: ICCVAM, skin sensitization, CPSC, DoD, FDA, NIST, NIOSH

Binding of a chemical to skin proteins is the first step in the development of allergic contact dermatitis. The electrophilic allergen screening assay is a chemical assay that measures light absorbance or a fluorescent signal in proportion to a chemical's tendency to bind to proteins.

The electrophilic allergen screening assay, developed by scientists at the National Institute for Occupational Safety and Health, was nominated to ICCVAM to evaluate its usefulness for identifying potential skin sensitizers. Four ICCVAM member agencies are participating in an ongoing validation study of the assay. Testing of 10 chemicals during 2018 showed that the method has sufficiently good reproducibility and accuracy rates to support further evaluation. In 2019, CPSC and the National Institute of Standards and Technology modified the assay to a 96-well format to increase throughput and accessibility of the assay; details of this process are described in an abstract (Gordon et al.) accepted for presentation at the SOT 2020 annual meeting. Testing of 20 chemicals will be conducted using the 96-well assay during 2020.

EpiAirway validation study

Tags: ICCVAM, NIEHS, inhalation toxicity, acute toxicity

Acute inhalation systemic toxicity tests identify substances that could cause illness or death after a single inhaled exposure. NICEATM facilitates interactions among stakeholder groups interested in developing and promoting alternatives to animal use for these tests.

A cooperative agreement under the NIEHS Phase IIb Small Business Innovation Research program provided funding to MatTek Corporation to validate its EpiAirway™ in vitro human bronchial tissue model to predict the toxicity of inhaled chemicals. Testing of reference chemicals to determine the usefulness and limitations of EpiAirway for this purpose has been completed. Several ICCVAM agency representatives are members of the cooperative agreement steering committee.

OptiSafe validation study

Tags: ICCVAM, NIEHS, eye irritation, acute toxicity

NICEATM coordinated a multi-laboratory validation study to determine the reliability and relevance of the OptiSafe test method. In this method, a test substance is applied to a semi-permeable membrane to assess the substance's potential to cause eye irritation.

The study was completed in 2018 and a report on the study has been accepted for publication (Choksi et al. 2020). The study demonstrated that the OptiSafe method is useful for identifying non-surfactant substances that do not require classification for ocular irritancy and thus can reduce the use of animals for this type of testing.

Review and comment on the Genomic Allergen Rapid Detection assay

Tags: ICCVAM, skin sensitization

The ICCVAM Skin Sensitization Workgroup reviewed the validation study report of the Genomic Allergen Rapid Detection™ assay for skin sensitization potential. This method measures changes in gene expression for 200 genes relevant to skin sensitization in a human myeloid leukemia cell line after the exposure of the cells to test substances.

The review of the validation study report started in February 2018. The ICCVAM workgroup did not support a further comprehensive assessment and peer review of this method, based on a number of considerations:

- Raw data from the study and subsequent nanoString analyses were not provided. A peer review cannot be conducted without access to raw data, necessary to evaluate the appropriateness and consistency of data analysis and processing.
- The prediction algorithm was not disclosed. This precluded a review of model features typically evaluated by peer review (e.g., statistical robustness of methods used).
- Eleven of the 28 chemicals used to develop the prediction model were used to evaluate the performance of the model. Chemicals used to develop the prediction model should not be used in an independent evaluation of model accuracy.
- The applicability domain of the method was not defined.

ICCVAM provided a response to test method developers in April 2018.

Evaluation of a predictive model of developmental vascular toxicity

Tags: EPA, NIEHS, NIH, developmental toxicity, adverse outcome pathways

NICEATM and EPA scientists built an AOP for quantitative prediction of developmental vascular toxicity. This AOP was then applied to ToxCast HTS data to develop predictions of chemicals' potential to disrupt angiogenesis, or blood vessel development (Saili et al. 2019). The predictions were evaluated for 38 compounds tested across a suite of functional assays for the angiogenic cycle, including assays in complex cell systems, virtual tissues, and small model organisms. The results serve to boost confidence in the capacity of HTS data to predict developmental vascular toxicity.

In vitro testing to support development of defined approaches for eye irritation of agricultural products

Tags: EPA, NIEHS, eye irritation, IATA, mixtures toxicity

NICEATM, the PETA International Science Consortium Ltd., EPA, and CropLife America member companies are collaborating to develop an in vitro defined approach for hazard classification of eye irritation potential of agrochemical formulations. A three-phased prospective evaluation was designed to (1) assess the applicability of seven in vitro eye irritation/corrosion protocols to agrochemical formulations and (2) develop a defined approach for agrochemical formulations testing for prediction of U.S. and international

irritancy classifications. In Phase 1, completed in 2018, six formulations were tested in seven different eye irritation test protocols. Ten additional agrochemical formulations with in vivo data representing a wider range of eye irritation classifications were evaluated in Phase 2 during 2019. While none of the methods directly correlated with the in vivo results, several methods showed potential for use in a defined approach. Analysis of the data from Phases 1 and 2 will determine the next steps of the study. An abstract describing this work (Choksi et al.) was accepted for presentation at the 2020 SOT annual meeting.

Validation of androgen receptor activity assays

Tags: NIEHS, endocrine disruptors, IATA

NICEATM collaborated with test method developer CertiChem, Inc. to validate an in vitro test method that uses MDA-Kb2 human breast cancer cells to measure androgen receptor agonist and antagonist activity. Specifically, NICEATM provided guidance on incorporating a cytotoxicity assay into the test method protocol. Testing of 67 coded reference chemicals in agonist and antagonist modes to characterize method reliability and relevance is finished, and a report summarizing these results was provided to CertiChem in December of 2018.

Collaboration with Cosmetics Europe to evaluate defined approaches for skin sensitization

Tags: NIEHS, skin sensitization, IATA

NICEATM and Cosmetics Europe collaborated to evaluate multiple defined approaches for skin sensitization safety assessment that had been submitted to OECD. The collaboration produced two publications:

- Hoffman et al. 2018 describes a database including data from human, animal, and five non-animal tests for 128 substances. The substances in the database have a variety of chemical structures and use categories. The database is a proposed point of reference for the evaluation and development of new non-animal approaches to skin sensitization safety assessment.
- Kleinstreuer et al. 2018 describes an analysis of multiple defined approaches for skin sensitization safety assessment of cosmetic ingredients using the database described in Hoffman et al. Many of these approaches were found to perform as well or better than animal methods to predict human skin sensitization hazards.

Based on the results of these analyses, NTP has adopted in vitro skin sensitization methods and associated defined approaches as the default approach to assess the potential for chemicals to cause allergic contact dermatitis.

Interlaboratory study to examine effects of key protocol elements for zebrafish developmental toxicity studies

Tags: NIEHS, developmental toxicity

To enable broader adoption of zebrafish for toxicological screening, NTP established the Systematic Evaluation of the Application of Zebrafish in Toxicology (SEAZIT) program.

An initial information-gathering phase of the SEAZIT program identified a need for an interlaboratory study to more closely examine the effects of variation in key protocol elements in developmental toxicity studies. This study began in 2019, and participating laboratories are conducting dose range-finding experiments. The study is designed to determine the effect of chorion removal and exposure media renewal on study outcomes. Participating laboratories will use in-house protocols to test a defined chemical set while varying the two protocol elements under investigation. The chemical set, which was designed to provide overlap with other NTP studies, includes chemicals with a range of physicochemical properties and developmental effects. Many of the chemicals have in vivo reference data available from rodent and other zebrafish studies. The interlaboratory study includes a pilot effort on chemical kinetics in support of future studies of absorption, distribution, metabolism, and excretion in zebrafish.

A primary goal of SEAZIT is to develop best practices for data analysis. To this end, the data generated in this study will be made publicly available, so that all study data may be used by investigators to estimate consensus toxicity values for each chemical.

Optimization and validation of an in vitro botulinum neurotoxin assay

Tags: NIEHS, DOI, biologics

Tests to detect and measure botulinum neurotoxin (BoNT) are required by multiple federal agencies for a variety of purposes, such as measuring toxin in drug formulations or detecting toxin in possibly contaminated food or wildlife. Currently, the standard test for these endpoints is a mouse lethality assay that can use large numbers of animals. NICEATM

supports efforts to develop, validate, and implement alternative approaches for tests used to detect the presence of botulinum neurotoxin and measure potency of BoNT preparations.

NICEATM supported the optimization and validation of enzyme linked immunosorbent assays (ELISA) that replace animal-based methods for diagnosing suspected avian botulism samples. Methods were developed for determining the presence or absence of BoNT serotypes C, D, and E in field-collected samples from a wide range of bird species. These methods may be used in the future to support testing requirements for the U.S. Geological Survey's National Wildlife Health Center.

Testing to expand the applicability domain of three in vitro skin sensitization assays

Tags: NIEHS, CPSC, EPA, FDA, skin sensitization, IATA

Defined approaches developed by NICEATM and ICCVAM use non-animal data to predict skin sensitization hazard and potency. These approaches combine data from the in vitro direct peptide reactivity assay (DPRA), KeratinoSens assay, and human cell line activation test (h-CLAT); read-across predictions generated by the QSAR Toolbox software package; and physical properties such as relative solubility in water and organic solvents. The defined approaches were described in papers published in 2016 and 2017 (Strickland et al. 2016, Strickland et al. 2017, Zang et al. 2017).

To assess and expand the potential applicability of these defined approaches to a broader range of chemical types, ICCVAM agencies nominated over 200 chemicals for additional testing in the DPRA, KeratinoSens, and h-CLAT tests. Chemicals being tested include pesticide ingredients and formulations, industrial chemicals, and personal care product ingredients. NTP is conducting this testing, which is expected to be completed in 2020. The study data will enable NICEATM and ICCVAM to evaluate the appropriateness of defined approaches using these three in vitro methods for various regulatory applications.

Addressing emerging contaminants

Tags: EPA, NIEHS, mixtures toxicity

The term NAMs refers to any non-animal technology, methodology, approach, or combination of these that can be used to provide information on chemical hazard and risk assessment. NAMs are being applied to address public concern about exposure and

environmental and health effects of emerging contaminants, driven in part by reports of potential human exposures to substances with limited information available on toxicity and exposure. Per- and polyfluoroalkyl substances (PFAS) are examples of such emerging contaminants. These substances present a complex problem, involving multiple chemicals, multiple routes of exposure, and multiple potential human health and ecological outcomes of concern. The hundreds of untested PFAS provide a scenario in which traditional one-by-one toxicity testing would require commitment of tremendous resources and assessment-relevant information would not be available for years. EPA and NTP are generating data through in vitro high-throughput toxicity testing and high-throughput toxicokinetic assays to inform hazard effects characterization and promote prioritization of chemicals for further in vivo testing (Patlewicz et al. 2019). This effort also will address PFAS lacking toxicity information by facilitating read-across approaches to infer the toxicological properties across the broader range of PFAS.

Evaluation of FXR-active chemicals identified from Tox21 screening

Tags: NIEHS, NIH, Tox21

Farnesoid X receptor alpha (FXRα) is a member of the nuclear receptor superfamily involved in bile acid homeostasis, glucose metabolism, lipid homeostasis, and hepatic regeneration. NIEHS and NIH scientists with academic and industry collaborators evaluated substances identified in Tox21 HTS in vitro screens as FXRα agonists and antagonists using four experimental approaches. The study generally confirmed quantitative HTS in vitro results, provided data on protein:protein interactions and receptor docking, and translated those results to an in vivo system. A poster describing the study (Hamm et al.) was presented at the 2018 SOT annual meeting.

Airman-on-a chip system applied to understanding hyperoxic oscillations

Tags: DoD, MPS, stem cells, neurotoxicity

AFRL is using iPSC technology to develop personalized brain-on-a-chip microfluidic platforms harnessing cells from individual airmen. These platforms will provide insight into how an airman's genetic background affects resiliency or susceptibility to operational conditions. The brain-on-a-chip platforms are comprised of iPSC-derived gluta- and gaba-minergic neurons, astrocytes, pericytes, and brain microvascular endothelial cells, and can

allow investigations of complex blood-brain barrier neurovascular interactions. In current studies, the platforms are exposed to dynamic oxygen conditions simulating a fighter pilot's oxygen exposure profiles, followed by comprehensive molecular analyses. In parallel, a matched human cohort is being evaluated in a hyperoxic chamber and physiologically and cognitively assessed. This study aligns in vitro work with in vivo human results to predict molecular outcomes and will provide a basis to predict an airman's resiliency and target enhancement strategies for optimal performance.

Caenorhabditis elegans assays for developmental neurotoxicity

Tags: FDA, developmental toxicity, neurotoxicity

Caenorhabditis elegans are small, non-pathogenic roundworms with many specialized tissues that function in ways that correspond to vertebrate organs. Many cellular and genetic pathways involved in development, neuronal architecture and function, and toxic mode of action are conserved between worms and humans (Hunt 2017). *C. elegans'* 3-day lifecycle and ease of maintenance suggest that the organism could be a good candidate model for fast and inexpensive alternatives to mammalian testing, but only if specific assays can be demonstrated to provide data that corresponds to mammalian toxic response. The worm development and activity test, developed by the FDA Center for Food Safety and Applied Nutrition, shows promise for identifying mammalian developmental neurotoxins (Hunt et al. 2018). The test is currently being assessed using a panel of 20 blinded compounds with known developmental and neurotoxicity effects in mammals.

Evaluation of a proposed approach to refine inhalation risk assessment for point of contact toxicity

Tags: EPA, inhalation toxicity

EPA conducts human health risk assessments to evaluate the potential health effects of pesticides and toxic chemicals based on the use pattern or conditions of use. For evaluating effects via the inhalation route, data are required from subchronic inhalation toxicity studies using animals, usually rats. However, human and rat respiratory tracts differ to an extent that may affect the ability of animal test results to correctly predict effects in humans. It is also challenging to accurately establish a no-observed-adverse-effect concentration from animal studies. To that end, Syngenta Crop Protection proposed using a 3D in vitro inhalation

toxicity model to derive a point of departure for inhalation toxicity for the fungicide chlorothalonil. The EPA Office of Pesticide Programs and Office of Pollution Prevention and Toxics provided feedback to Syngenta during development of the proposed approach. Results of the case study were evaluated by the Scientific Advisory Panel for the Federal Insecticide, Fungicide, and Rodenticide Act at their December 2018 meeting. The panel's report, issued in April 2019, expresses support for the approach and makes recommendations for improvements. EPA has continued to work with Syngenta to address the panel's recommendations.

Using ToxCast data for food chemical safety risk assessment

Tags: EPA, FDA, IVIVE

NAMs are currently being developed and evaluated for use in chemical safety risk assessment, including chemicals used in food. NAMs include in vitro HTS assays such as those used in ToxCast and Tox21. These assays have been run for thousands of compounds, including hundreds of compounds used in food. However, the relationship of these NAM data with traditional in vivo animal data and the utility of NAMs for risk assessment remain under evaluation. This study evaluated the utility of ToxCast/Tox21 HTS data in food safety risk assessment. To do this, bioactive concentrations of a subset of food-use compounds in ToxCast were converted to oral equivalent doses via IVIVE using either in vitro or in silico-based toxicokinetic parameters for a subset of food-use compounds. These oral equivalent doses were then compared to doses demonstrated to cause effects in in vivo animal tests using data compiled by EPA and FDA. Initial comparisons demonstrated great variability in the correlation between ToxCast and in vivo data, so steps are being taken to further refine the toxicokinetic information, chemical groups, and in vivo endpoints to identify additional information and conditions necessary to utilize HTS data for preliminary food safety assessment.

Communication and Education

The Strategic Roadmap identifies stakeholder engagement as critical to acceptance and use of new methods. To facilitate stakeholder engagement, ICCVAM and its member agencies organize public meetings and webinars. These events inform stakeholders about the

availability and appropriate use of new methods and provide opportunities for stakeholders and agencies to discuss opportunities for test method development.

ICCVAM Communities of Practice webinars

Tags: ICCVAM, inhalation toxicity

In 2015, ICCVAM initiated a series of Communities of Practice webinars to provide opportunities for detailed presentations on a current topic relevant to alternative test method development.

- The fourth of these webinars, presented on Jan. 23, 2018, was titled Machine Learning in Toxicology: Fundamentals of Application and Interpretation. The webinar, viewed by nearly 200 people, explored the fundamentals of machine learning approaches, including how they work, how they are interpreted, and precautions that should be taken when evaluating their output. Sean Ekins, Ph.D., D.Sc., Collaborations Pharmaceuticals, Inc., and NICEATM Deputy Director Nicole Kleinstreuer, Ph.D., addressed issues specific to machine learning approaches used in a regulatory context. Case studies were presented to highlight where such techniques have been applied successfully both nationally and internationally.
- Nearly 250 viewers attended the Jan. 22, 2019, Communities of Practice webinar, Non-animal Approaches for Inhalation Toxicity Testing. Amy Clippinger, Ph.D., PETA International Science Consortium Ltd., provided an overview of several ongoing collaborative efforts associated with acute and subchronic inhalation toxicity testing, with emphasis on available human cell-based and in silico methodologies, considerations of dosimetry, and development of technologies that could provide predictive test methods in the future. Paul Hinderliter, Ph.D., DABT, Syngenta Crop Protection, Inc., presented a case study describing a source-to-outcome approach that uses in vitro and in silico methods to refine inhalation risk assessment for point of contact toxicity.

ICCVAM Public Forums

Tags: ICCVAM, acute toxicity

ICCVAM held two public forum meetings in 2018 and 2019. These annual meetings provide an opportunity for public interaction with representatives from ICCVAM member agencies.

- View materials from the 2018 Public Forum
- View materials from the 2019 Public Forum

ICCVAM held its fifth public forum on May 24, 2018, at NIH in Bethesda, Maryland. This meeting was attended by about 100 individuals in person and remotely, who heard presentations by ICCVAM members on current activities related to the development and validation of alternative test methods and approaches. Seven ICCVAM member agencies provided updates on activities. Other presentations described FDA and EPA strategic plans to promote the use of non-animal methods and the updated Tox21 strategic plan and goals for the program's next five years. A major focus of this meeting was implementation of the Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States. Commenters offered suggestions on specific actions that should be taken to advance the goals of the Strategic Roadmap and encouraged agencies that have been less active in ICCVAM activities to take a greater role.

The 2019 public forum was held on May 23 at NIH in Bethesda. About 40 public participants and over 200 webcast viewers heard presentations by ICCVAM members representing eight U.S. agencies on current activities related to the development and validation of alternative test methods and approaches, many of which addressed goals of the Strategic Roadmap. A key focus was progress made towards reduction and replacement of animal use for acute toxicity tests required by regulatory agencies: acute systemic toxicity, skin and eye irritation, and skin sensitization testing. Public comments submitted to the meeting praised specific actions agencies had taken to advance the Strategic Roadmap goals and suggested additional activities that could support further progress.

ICCVAM advisory committee meetings

Tags: ICCVAM, MPS

The Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) is a federally chartered advisory group that advises NICEATM, ICCVAM, and the Director of NIEHS about ICCVAM activities. SACATM held public meetings on Sept. 5-6, 2018, at

NIEHS in Research Triangle Park, North Carolina and Sept. 19-20, 2019, at the Crowne Plaza Crystal City in Arlington, Virginia.

The focus of SACATM's September 2018 meeting was actions needed to advance goals outlined in the Strategic Roadmap. Industry representatives stated a desire for clear direction from regulators about their information needs and for communication by regulators of their willingness to accept data from new approach methodologies to fulfill those needs. Participants discussed the need for high-quality reference data from past animal tests to evaluate the performance of new methods and also considered issues involved in sharing and using those data.

Presentations at the September 2019 meeting focused on new approaches to validation, computational tools, and applications of MPS. SACATM members expressed support for the current activities and direction of ICCVAM and noted the progress that had been made in advancing alternatives to animal testing. Discussions on the use of computational methods focused on the limitations and applications of machine learning models in predicting toxicity. Considering the potential uses of MPS in predicting human toxicity, committee members suggested these systems might be most useful for screening early-stage toxicity, evaluating effects on diverse populations, and providing models for applications lacking established animal models. They cautioned, however, that the context of use for these platforms would need to be clearly defined.

ICCVAM agency-sponsored workshops and webinars

Tags: EPA, FDA, NIEHS, acute toxicity, MPS, biologics

ICCVAM agencies convened workshops and webinars during 2018 and 2019, summarized in the table below, to foster collaboration and provide information about alternative testing methods.

Meeting Date and Location	**Sponsoring Agency (and partner organization)**	**Meeting Title (with link to page if available)**	**Meeting Summary**
March 27, 2018-Dec.12, 2019	FDA (Society of Toxicology)	Colloquia on Emerging Toxicological Science: Challenges in Food and Ingredient Safety	Since 2014, SOT and the FDA Center for Food Safety and Applied Nutrition have presented colloquia on high-quality, cutting-edge, future-

Meeting Date and Location	Sponsoring Agency (and partner organization)	Meeting Title (with link to page if available)	Meeting Summary
			oriented toxicological science topics. Many of the colloquia focus on alternatives to animal use. The series is continuing into 2020.
Apr. 11-12, 2018	NICEATM	Predictive Models for Acute Oral Systemic Toxicity	This workshop presented in silico models to predict acute oral toxicity endpoints of regulatory interest. Participants also discussed developing a consensus model to integrate the submitted models, as well as next steps to encourage the appropriate use of these models in regulatory contexts.
Aug. 9, 2018	FDA	What We're Doing to Advance In Silico Medicine at FDA	The webinar provided an overview of in silico modeling and simulation approaches used by FDA's Office of Device Evaluation.
Sept. 10, 2018	FDA	Human Dermal Safety Testing for Topical Drug Products	This workshop addressed the current state and future directions for collection of human data on potential skin toxicity of topically applied medications. Participants considered the impact of human skin toxicity studies on drug labeling and discussed alternative approaches to providing information about skin toxicity.
Sept. 18-19, 2018	NICEATM (PETA International Science Consortium Ltd.)	The Monocyte Activation Test for Pyrogen Testing of Medical Devices	The workshop focused on use of the monocyte activation test as a standalone release test to replace animal use when satisfying biocompatibility and sterility testing requirements for medical devices.
Sept. 27, 2018-Dec. 6, 2018	NICEATM	The Utility of Zebrafish Models for Toxicology	This webinar series examined three case studies

Meeting Date and Location	**Sponsoring Agency (and partner organization)**	**Meeting Title (with link to page if available)**	**Meeting Summary**
			that illustrate the utility of zebrafish models for toxicology.
Oct. 16-17, 2018	NICEATM (International Alliance for Biological Standardization – North America)	Implementing Nonanimal Approaches for Human and Veterinary Vaccine Testing: Achieving Scientific and Regulatory Success for Rabies and Beyond	This workshop brought together scientific and regulatory leaders from government, academia, and industry to develop recommendations for advancing alternative methods for human and veterinary rabies vaccine testing.
Nov. 7, 2018-Nov. 13, 2019	EPA (PETA International Science Consortium Ltd., Physicians Committee for Responsible Medicine)	Webinar Series on the Use of New Approach Methodologies (NAMs) in Risk Assessment	These webinars discussed skin sensitization testing, dosimetry modeling of inhaled substances, respiratory sensitization, and fish toxicity. The series will continue in 2020.
March 6, 2019	NIEHS and EPA	Converging on Cancer	This workshop aimed to provide a clear path forward for evaluating the interactions between environmental exposures and cancer biology using the latest tools in toxicology and identifying knowledge gaps that require research attention.
May 2, 2019	FDA	Decision-making in Non-animal Cosmetic Safety Assessment	The webinar introduced a new stakeholder collaboration with the goal of globally implementing non-animal cosmetic safety assessment by 2023. Webinar speakers discussed applicable risk assessment principles and relevant case studies.
May 3, 2019	EPA (ASCCT, ESTIV)	New Computational Tools from EPA	EPA scientists provided overviews of the CompTox Chemicals Dashboard and the EPA's generalized read-across (GenRA) application.

Meeting Date and Location	**Sponsoring Agency (and partner organization)**	**Meeting Title (with link to page if available)**	**Meeting Summary**
May 14-31, 2019	NIEHS and EPA	New Approaches and Alternatives for Toxicity Testing	The NIEHS Superfund Research Program hosted a webinar series highlighting research that may be useful as new approaches and methodologies for toxicity testing.
June 6-7, 2019	NIEHS (National Academies of Science, Engineering, and Medicine)	Leveraging Artificial Intelligence and Machine Learning to Advance Environmental Health Decisions	This workshop explored emerging applications and implications of artificial intelligence and machine learning in environmental health research. Speakers highlighted the use of these technologies for characterizing sources of pollution, predicting chemical toxicity, estimating human exposures to contaminants, and identifying health outcomes.
Oct. 24-25, 2019	NIEHS (National Institute of Dental and Craniofacial Research; National Heart, Lung, and Blood Institute; National Institute of Arthritis and Musculoskeletal and Skin Diseases)	Tissue Chip Platforms as Tools for Testing Biocompatibility and Biotoxicity of Biomaterials	Topics discussed included the design and validation of tissue chip platforms for assessing biomaterial properties, and applying tissue chip platforms to prediction of in vivo responses of tissues and organs to biomaterials
Oct. 30-31, 2019	NICEATM (Physicians Committee for Responsible Medicine)	Mind the Gaps: Prioritizing Activities to Meet Regulatory Needs for Acute Systemic Lethality	Participants in this workshop considered approaches available for assessing acute lethality associated with chemicals and chemical mixtures, with the goal of designing comprehensive strategies to predict toxicity while avoiding animal tests.
Dec. 3, 2019	NICEATM (PETA International Science Consortium Ltd.)	Developing Strategies to Increase the Use of Recombinant Antibodies	Experts from academia, biotechnology, government, and animal welfare organizations discussed using non-animal derived antibodies in research, diagnostics, and biotechnology.

Meeting Date and Location	Sponsoring Agency (and partner organization)	Meeting Title (with link to page if available)	Meeting Summary
Dec. 17, 2019	EPA	First Annual Conference on the State of the Science on Development and Use of New Approach Methods (NAMs) for Chemical Safety Testing	Experts from EPA, other government agencies, academia, and industry discussed application of NAMs to a variety of toxicity areas. Breakout groups considered topics including reference data and building scientific confidence.

Publication of summary of current zebrafish husbandry and toxicology study practices

Tags: NIEHS, developmental toxicity

A 2019 paper (Hamm et al. 2019) summarized the initial SEAZIT information-gathering efforts. Investigators in academic, government, and industry laboratories that routinely use zebrafish embryos for chemical toxicity testing were asked about their husbandry practices and standard protocols. Information was collected about protocol components including zebrafish strains, feed, system water, disease surveillance, embryo exposure conditions, and endpoints. Literature reviews assessed issues raised by the investigators. Interviews revealed substantial variability across design parameters, data collected, and analysis procedures. The presence of the chorion and renewal of exposure medium (static versus static-renewal) were identified as design parameters that could potentially influence study outcomes and are being addressed in an ongoing interlaboratory study. The information gathered in this effort provided a basis for future SEAZIT activities to promote more consistent practices among researchers using zebrafish embryos for toxicity evaluation.

Update of ToxTutor

Tags: NLM, adverse outcome pathways, stem cells

NLM updated its online ToxTutor course in August 2018. ToxTutor is a self-paced tutorial that covers the key principles of toxicology and related topics. It provides information on alternatives to animal testing and the state-of-the-science approaches for risk assessments. If a certificate of completion is needed, ToxTutor can be completed through a learning management system. ToxTutor is being used in academic courses and for training in

companies and elsewhere. It is approved by some organizations for continuing education contact hours.

The 2018 update of ToxTutor added "The Microbiome" as a new topic, expanded content on alternatives to animal testing and human-on-a-chip testing approaches, and included induced pluripotent stem cells as an emerging approach for toxicity testing. The tutorial also added more content on AOPs and new sections on "Intuitive Toxicology and Risk Communication" (including content on uncertainty) and "Environmental Toxicology, Environmental Health, and One Health."

In December 2019, NLM made changes to web resources as part of the reorganization associated with their current strategic plan. These changes could cause ToxTutor to be relocated in the future. NLM will be conducting webinars and other presentations in 2020 to inform users how they can continue to access web resources.

Animal Welfare Information Center resources

Tags: USDA, metrics

The 1985 amendments to the Animal Welfare Act established the Animal Welfare Information Center (AWIC), an information service at the U.S. Department of Agriculture (USDA) National Agricultural Library. AWIC's mission is to provide information pertinent to employee training that could prevent unintended duplication of animal experimentation and on improved research methods that could reduce or replace animal use and minimize pain and distress to animals.

AWIC provides information to the toxicology community on the National Agricultural Library website related to the development and use of alternatives to toxicity testing, as well as information and guidelines on mandatory and regulatory testing set by various agencies. These web pages highlight peer-reviewed publications and other online resources that discuss ways to replace, reduce, and refine animal use in toxicity testing. For example, AWIC provides links and citations on biologics and vaccine testing topics such as *Leptospira* vaccine potency testing.

During 2018 and 2019, 314,831 people visited the AWIC website. These visits generated a total of 677,149 page views. AWIC also provides information through outlets such as

presentations and posters, workshops, webinars, and conference exhibits. Through these outlets, AWIC presented to over 825 participants at 22 different outreach events in 2018 and 2019, collectively. At these outreach events, AWIC described best practices for searching and finding current literature on methods to reduce, refine, or replace animal use in testing and experimentation.

Computational Tools Applications

ICCVAM and its member agencies are exploring how computational approaches can be applied to reduce animal use for toxicity testing. These approaches have potential application for acute oral toxicity and eye irritation testing, and for predicting whether chemicals could cause cardiotoxicity, developmental toxicity, or neurotoxicity.

In silico screening approaches for assessing cardiovascular safety

Tags: NIEHS, cardiotoxicity

NIEHS scientists are using two approaches to develop computational models for predicting the potential of substances to cause toxicity to the heart and vascular system.

In one approach, a weighted gene coregulation network analysis was applied to rat heart gene expression data from the DrugMatrix database to map gene expression relationships associated with cardiotoxic stress. The map revealed sets of genes linked to biological processes with potential relevance to cardiotoxicity. Applied to known cardiotoxic substances such as anthracyclines, corticosteroids, and kinase inhibitors, the map may provide an approach for exploring mechanisms of cardiotoxicity. An abstract describing this project (Rahman et al.) was accepted for presentation at the SOT 2020 annual meeting.

The second approach used in silico tools and in vitro HTS data to generate bioactivity scores for environmental chemicals against molecular and cellular targets known to mediate bioactivity in the cardiovascular system. These scores support a visualization tool and ranking system that can be used to screen and prioritize chemicals with limited or no toxicity information for further assessment. An abstract describing this project (Krishna et al.) was accepted for presentation at the SOT 2020 annual meeting.

Statistical models for classification of eye irritants

Tags: NIEHS, eye irritation, mixtures toxicity

NICEATM developed statistical models that could potentially be used to classify chemicals as eye corrosives, irritants, or non-irritants according to EPA and GHS hazard classification endpoints. Models were developed using machine learning approaches combined with historical in vivo eye irritation data, chemical structural information, and physicochemical properties. These models were used to predict hazard classifications for a database of over 500 substances, including many mixtures. Results suggest that these models are useful for screening substances for eye irritation potential. Future efforts to increase the models' utility will focus on expanding their applicability domains and using the models in conjunction with other input variables in a defined approach for eye irritation testing. A paper describing this work is in preparation.

Adverse outcome pathway for embryonic vascular development

Tags: EPA, NIEHS, developmental toxicity, adverse outcome pathways

Work to identify alternative methods for developmental toxicity testing has focused on understanding and predicting disruption of key mechanisms in embryonic and fetal development. AOPs provide a useful framework for integrating the evidence derived from in silico and in vitro systems to inform chemical hazard characterization. An ongoing collaboration between NICEATM and EPA has built and applied an AOP for developmental toxicity through a mode of action linked to embryonic vascular disruption. A 2019 publication (Saili et al. 2019) reviewed the model for quantitative prediction of developmental vascular toxicity from ToxCast HTS data and compared the HTS results to functional vascular development assays in complex cell systems, virtual tissues, and small model organisms. Results increased confidence in the capacity to predict adverse developmental outcomes from HTS in vitro data and model computational dynamics for in silico reconstruction of developmental systems biology.

Additivity approaches to predicting toxicity of formulations

Tags: EPA, NIEHS, acute toxicity, oral toxicity, mixtures toxicity, inhalation toxicity

The EPA Office of Pesticide Programs has been accepting voluntary submissions of oral and inhalation toxicity data for agrochemical formulations under a pilot program to evaluate the usefulness and acceptability of a mathematical tool that estimates the toxicological classification of a chemical mixture. The submitted data were paired with toxicity

calculations done in accordance with the GHS additivity equation based on the individual components of the formulation. NICEATM is evaluating the extent to which predictions of acute toxicity for a formulation derived using the additivity equation compare to in vivo test results. The evaluation will be finalized in 2020 along with a report describing the results.

CATMoS and additivity approaches to predict toxicity of mixtures

Tags: DoD, acute toxicity, oral toxicity, mixtures toxicity

While exposure of humans to environmental hazards often occurs with complex chemical mixtures, most existing toxicity data and tools are for single compounds. An approach to estimating toxicity of mixtures is provided by the GHS additivity formula, which is based on the acute toxicity estimate of ingredients. The concentration-addition method assumes that all components in the mixture share the same mechanism of toxicity and the toxicity of the mixture is sum of their concentration and potency. Air Force researchers used data in ICE for assessment of acute oral toxicity of mixtures. The ICE database contains in vivo acute oral toxicity data for about 10,000 chemicals and more than 500 mixtures. By using the available experimental data for single compounds, the GHS category could be calculated for 273 mixtures. Use of CATMoS predictions available via OPERA enabled toxicity estimates for 487 mixtures with 69% accuracy for GHS classification. For 172 mixtures with two or more active ingredients, the accuracy rate was 78%. These results demonstrate that CATMoS together with the additivity formula can be used to predict GHS category for chemical mixtures.

Use of a PBPK model to derive a human-equivalent dose

Tags: DoD, neurotoxicity

CCDC CBC has successfully used PBPK modeling to derive a high-fidelity human-equivalent dose of the ultra-potent opioid carfentanil. This effort included validating the pharmacokinetics of carfentanil in an in vivo rabbit model with the rabbit PBPK in silico model, converting to a human physiology of interest, and then calculating an equivalent dose by optimizing the maximal plasma concentration and area under the curve of the PK profile. CCDC CBC's predicted human-equivalent lethal dose of carfentanil differed from that of the U.S. Drug Enforcement Administration by only 50 ng. CCDC CBC's PK profile is also supported by a Canadian report of an overdose of carfentanil by inhalation administration in

which periodic blood samples were analyzed for carfentanil concentration from admission into the emergency department until discharge.

Current efforts aim to convert this intravenous human equivalent dose into an inhaled dose by using the PBPK model's pulmonary administration module. Additionally, CCDC CBC aims to predict dermal absorption in a reliable way using the transdermal module of their PBPK software suite.

Utilization

Federal agencies can play an important role in facilitating the successful adoption and use of NAMs, both within the United States and internationally. ICCVAM agencies strive to provide clear guidance on the use and acceptance of data from new methods. Where possible, they implement approaches to reduce and replace animal use.

Assessments of Agency Needs and Practices

Identifying agencies' testing needs is a key step towards implementation of the Strategic Roadmap. In 2018, ICCVAM workgroups published papers describing agency testing requirements for the "six-pack" of acute toxicity tests, including acute systemic toxicity, skin and eye irritation, and skin sensitization. A 2019 paper outlined use and acceptance of read-across, and efforts are ongoing to define testing needs for ecotoxicity.

Publication of paper outlining agency needs and applications for read-across

Tags: ICCVAM, EPA, FDA

Read-across is a computational technique that uses toxicity data from data-rich chemicals to predict toxicity for an untested or data-poor chemical. Despite the potential usefulness of read-across to provide information on chemical hazard and risk assessment, its application and acceptance varies among U.S. federal agencies. ICCVAM established a read-across workgroup sponsored by EPA and FDA that conducted a survey of the current applications, tools used, and needs of the agencies represented in the workgroup. Of the agencies surveyed, EPA had the greatest experience in using read-across. However, other agencies indicated that they would benefit from gaining a perspective of the landscape of available tools and guidance. The paper summarizing the survey (Patlewicz et al. 2019) also provided practical case studies to illustrate how the read-across approaches applied by two agencies varied based on agency decision contexts.

Publication of paper outlining agency testing needs in acute systemic toxicity

Tags: ICCVAM, acute toxicity, oral toxicity, inhalation toxicity

Understanding the current regulatory use and acceptance of non-animal data is a necessary starting point for future method development, optimization, and validation efforts. Therefore, the ICCVAM Acute Toxicity Workgroup reviewed (Strickland et al. 2018) acute systemic

toxicity testing requirements for six U.S. agencies and noted whether there is flexibility in satisfying data needs with methods that replace or reduce animal use. The review informed the development of the Strategic Roadmap for implementing non-animal approaches to assess potential hazards associated with acute exposures to industrial chemicals and medical products.

Publication of paper outlining agency testing needs in skin and eye irritation

Tags: ICCVAM, eye irritation, skin irritation

Chemical regulation authorities in the United States require or consider eye and skin irritation test data to develop product hazard labeling or to assess risks for exposure to skin- and eye-irritating chemicals. The ICCVAM Ocular and Dermal Irritation Workgroup reviewed U.S. agencies' data needs for skin and eye irritation (Choksi et al. 2019). Information reviewed included the type of skin and eye irritation data required by each agency and the associated decision context: hazard classification, potency classification, or risk assessment; the preferred tests; and whether alternative or non-animal tests are acceptable. Information on the specific information needed from non-animal test methods was also collected. The review revealed a willingness to consider non-animal or alternative test methods by participating agencies, who encouraged sponsors to consult with the relevant agency to discuss the use and acceptance of alternative methods for local skin and eye irritation testing in designing their testing program.

Summaries of U.S. and international testing needs in skin sensitization

Tags: ICCVAM, skin sensitization

U.S. regulatory and research agencies often rely upon skin sensitization test data to assess the sensitization hazards associated with dermal exposure to chemicals and products and ensure that such substances will not cause unreasonable adverse effects to human health when used appropriately. The ICCVAM Skin Sensitization Workgroup conducted a review of U.S. agencies (Strickland et al. 2019) to identify the standards, test guidelines, or guidance documents that are applicable to satisfy each of these agency's needs; the current use of animal testing and flexibility for using alternative methodologies; information needed from alternative tests to fulfill the needs for skin sensitization data; and whether data from non-animal alternative approaches are accepted by these agencies.

NICEATM scientists and International Cooperation on Alternative Test Methods (ICATM) participants also reviewed skin sensitization testing requirements for ICATM participating countries or regions (Daniel et al. 2018). The survey considered the type of skin sensitization data required for each chemical sector and whether these data were used in a hazard classification, potency classification, or risk assessment context; the preferred tests; and whether alternative non-animal tests were acceptable. An understanding of national and regional regulatory requirements for skin sensitization testing will inform the development of ICATM's international strategy for the acceptance and implementation of non-animal alternatives to assess the health hazards and risks associated with potential skin sensitizers.

Survey of information needs for ecotoxicity

Tags: ICCVAM, DoD, DOI, EPA, ecotoxicity

While the Strategic Roadmap focuses primarily on human health, ICCVAM also recognizes the need to implement non-animal approaches for ecotoxicity testing. Development and implementation of alternative approaches to reduce and replace animal use for this testing will involve four key steps: (1) defining testing needs, (2) identifying the available alternative approaches, (3) developing defined approaches to testing and assessment, and (4) addressing both scientific and non-scientific (including regulatory) challenges. Accordingly, in 2018 ICCVAM established an ecotoxicology workgroup to provide expertise in identifying and evaluating alternative approaches to identify ecological and environmental hazards using in vitro and/or in silico methods. The workgroup is sponsored by DoD, DOI, and EPA and has representatives from seven ICCVAM agencies. Members are currently compiling a summary of ecotoxicity tests that require the use of animals, emerging technologies for ecotoxicity and environmental safety, and the utility of those technologies in regulatory testing. This summary, planned for publication in 2020, will help advance the development and evaluation of defined approaches for screening, testing, and assessment of relevant endpoints.

Exploring utility of IVIVE in risk assessment and regulatory decision-making

Tags: ICCVAM, ATSDR, EPA, IVIVE

IVIVE can facilitate the use of in vitro toxicity testing data in risk assessment and regulatory decision-making. In 2018, ICCVAM established an IVIVE workgroup to explore how IVIVE can best be applied to these purposes. The workgroup is sponsored by ATSDR and EPA, and

currently has members from seven ICCVAM agencies. The workgroup is currently cataloging and evaluating currently available IVIVE approaches. Activities have focused on harmonizing the technical terms used in IVIVE application, evaluating their suitability for specific research or regulatory purposes, and assessing whether additional tools or models are needed. The workgroup's findings will be reported in a publication planned for 2021 that will systematically present the best practices and encourage the judicious use of IVIVE.

Survey of information needs for nanomaterials

Tags: ICCVAM, CPSC, NIST

Nanomaterials are used in a broad range of consumer products, but their unique properties make them challenging to test in both in vivo and in vitro toxicity assays. ICCVAM established its Nanomaterials Workgroup to evaluate the suitability of alternative methods to assess potential toxicity of nanomaterials. The workgroup is sponsored by CPSC and the National Institute of Standards and Technology, and has members from seven ICCVAM agencies. The workgroup has surveyed federal agencies to determine needs and activities relevant to nanomaterials testing, potential applicability of alternative methods to testing needs, and the challenges that would be faced in implementing them. Its findings will be reported in a paper planned for publication in 2020.

Request for information on botulinum neurotoxin assays

Tags: NIEHS, biologics

Tests to detect and measure BoNT are required by multiple federal agencies for regulatory and other decision contexts. Currently, the standard test for these endpoints is a mouse lethality assay that can use large numbers of animals. In a June 2018 Federal Register notice (83 FR 27622), NICEATM requested available data and information on approaches and/or technologies currently used to detect and measure BoNT. The scope of the request included information on any activities relevant to the development or validation of alternatives to in vivo test methods currently used by federal agencies for regulatory and other decision contexts. The announcement also requested available data from in vivo BoNT tests used for similar applications as the proposed alternative, such as distinguishing between BoNT serotypes in biological matrix samples or measuring the potency of therapeutic BoNT preparations. Submitted information is being used to assess the state of the science and

determine technical needs for non-animal test methods that are used to detect the presence of BoNT and measure potency of BoNT preparations.

Request for information on developmental toxicity assays

Tags: NIEHS, developmental toxicity

Developmental toxicity tests evaluate the extent to which exposure to a substance can interfere with normal development. This testing is required by multiple regulatory agencies and uses large numbers of animals. In a May 2018 Federal Register notice (83 FR 20082), NICEATM requested available data and information on approaches and/or technologies currently used to identify potential developmental toxicants. Submitted information has been used to assess the state of the science for these approaches and technologies and determine technical needs for approaches to assess this endpoint.

Initiatives to Replace or Reduce Animal Use

Reducing or eliminating animal testing is a key goal of the Strategic Roadmap. To this end, ICCVAM agencies that conduct chemical safety testing develop and use approaches that reduce or replace animal use. Likewise, ICCVAM agencies that require their stakeholders to conduct testing are reducing required animal use by developing criteria for waiving tests.

Alternative criteria for classification of corrosive materials

Tags: DOT, skin irritation, eye irritation

The U.S. Department of Transportation (DOT) proposed an amendment to the Hazardous Materials Regulation in November 2018 (83 FR 60970). The amendment would enable non-testing alternatives that use existing data on chemical properties to be considered for classifying corrosive mixtures. Currently, the regulation requires offerors to classify Class 8 corrosive material and assign a packing group based on test data. The regulation authorizes a skin corrosion test and various in vitro test methods that do not involve animal testing. However, only data obtained from testing are currently acceptable for classification and assignment to a packing group. These alternatives would afford offerors the ability to make a classification and packing group assignment without the need to conduct physical tests.

Integrated screening process for potential fish toxicants

Tags: DOI, acute toxicity, ecotoxicity

Fisheries managers use toxicants to control invasive and undesirable fish species. The U.S. Geological Survey (USGS) Upper Midwest Environmental Science Center of the U.S. Department of the Interior (DOI) developed a screening process to minimize and replace animal testing while developing new chemical control agents for invasive species. The goal is to identify compounds that are potentially toxic to target species while posing minimal risk to native species. The three integrated phases of the screening process include (1) identifying physical and chemical properties of compounds that affect bioavailability in fish, (2) prescreening of a publicly available chemical databank to prioritize candidate compounds, and (3) screening of selected compounds for cytotoxicity using in vitro biological assays, ecotoxicity modeling, and fish cell lines. Although in vivo testing continues to be utilized in the development of new fish toxicants, the screening process enables minimization of animal testing while developing a new chemical control agent for invasive species.

Ecotoxicity modeling uses a QSAR modeling system that determines species-specific responses to chemical exposures using existing toxicity data and chemical properties. These in silico assessment methods can be used to prioritize candidate compounds and estimate cytotoxicity. Models can predict more than 3,000 endpoints for over 600 taxa against more than 9,000 chemicals. A user interactive prototype of the model results is available on the USGS website.

Promising novel toxicants identified in the ecotoxicity modeling step are tested using cellular assays to identify which toxicants have the biological activity required. USGS has developed multiple endpoint assays that measure cell viability based on quantitation of adenosine triphosphate. These assays use cell lines from native species including fathead minnow, bluegill sunfish, rainbow trout, lake sturgeon, and paddlefish, as well as invasive species, including silver carp and bighead carp. Six novel species-selective toxicants have been identified. Four toxicants demonstrating potent species-selective cytotoxic effects are being manufactured for in vivo toxicity screening assays. Two toxicants have completed in vivo testing and show modest selectivity for silver and bighead carp.

Use of a fish embryo toxicity test for prioritizing testing of environmental contaminants

Tags: DOI, acute toxicity, ecotoxicity, cardiotoxicity, developmental toxicity

As part of ongoing assessments of wildlife health, DOI is investigating potential cardiovascular effects on fish from pesticides and pharmaceuticals frequently detected in surface waters and fish tissues. The USGS Columbia Environmental Research Center conducts high-content screening of compounds to formulate hypotheses and prioritize compounds for further toxicity testing. This approach reduces animal use, test compound needed, and waste by utilizing pre-feeding fish embryos in a microtiter plate format. This approach is also being used to better characterize toxicity of polycyclic aromatic hydrocarbons (PAHs) and oxygenated PAHs in groundwater samples over different trophic levels of a subsurface oil spill. These assays can provide evidence to justify larger-scale studies to determine actual risk versus perceived risk of contaminants.

The Center's current high-content screening assay is a developmental cardiotoxicity assay that assesses total body length, pericardial area, intersegmental vessel area, circulation, and heart rate after a 72-hour exposure. This array of endpoints allows for a targeted assessment of toxicity. In addition to an LC50 estimate, the assay rapidly provides mode-of-action information, allowing formation of hypotheses on sublethal impacts of contaminants. Data derived from these studies on acute toxicity and mode of action for pesticides, pharmaceuticals, and PAHs/oxygenated PAHs will support a better understanding of potential effects on wildlife species.

High-throughput in vitro assays to identify environmental contaminants

Tags: DOI, ecotoxicity, endocrine disruptors

DOI utilizes cell-based bioassays for HTS of contaminants in environmental samples. The cell-based bioassays at the USGS Columbia Environmental Research Center are responsive to dioxin-like chemicals, PAHs, and a variety of endocrine-active substances such as estrogenic, androgenic, glucocorticoid-like, progestogenic, and steroidogenic chemicals. HTS is a valuable tool for quickly and inexpensively identifying bioactive samples for further study in higher-tier assays. DOI utilizes these assays for mapping PAH-like activity in oil-contaminated groundwater, mapping estrogenic activity in sediments from the Upper Coosa River Basin, quantitating estrone in laboratory water samples, assessing fish feed for estrogenic activity, testing pure chemicals and chemical mixtures for bioactivity, and guiding chemical analysis of endocrine-active chemicals in an effects-directed analysis of sediments

and water from the Chesapeake Bay watershed. Currently, nontargeted analysis of bioactive fractions from the Chesapeake Bay watershed sediments is underway for identification of endocrine-active chemicals. A similar approach will be used to identify oxygenated PAHs from oil-contaminated groundwater. Several of the cell bioassays are also undergoing mechanistic evaluation for expanded utility in screening additional types of contaminants. The cell bioassays at the Center are primarily luciferase transactivation cell bioassays and include cell lines from the EPA's Endocrine Disruptor Screening Program and Tox21.

In vitro methods and molecular profiling to evaluate effects of cyanobacteria toxin in fishes

Tags: DOI, ecotoxicity

Cyanobacteria, also known as blue-green algae, produce potent toxins called microcystins. During algal blooms, concentrations of microcystins can reach levels that are toxic to vertebrates and adversely affect fish and wildlife health. While acute effects of high-dose microcystin exposure have been investigated, there has been less focus on adverse effects resulting from low-dose or chronic exposure. In addition, there are few options for testing effects on non-model organisms. During 2018-2019, scientists in the USGS of the DOI developed and applied methods to evaluate cellular and molecular responses to microcystins using primary tissue culture approaches. These in vitro methods include evaluation of primary hepatocytes and leukocytes from fishes that inhabit aquatic ecosystems vulnerable to harmful algal blooms. Specifically, this research focused on smallmouth bass collected from the upper Chesapeake Bay watershed. This analytical approach includes the application of in vitro exposures, image analysis-based flow cytometry, and transcriptional profiling of hepatic and immune-responsive genes using nCounter technology. These in vitro approaches, used to interrogate specific mechanistic questions in environmentally relevant fishes, minimize the use of vertebrates.

Alternative approaches to evaluating bioactivity in surface waters

Tags: DOI, ecotoxicity, endocrine disruptors, acute toxicity

Chemical contaminants are introduced to environmental waters via many sources, and many of these contaminants have the potential to adversely affect organisms living in these waters. Recognized adverse effects include the induction of cancer via genotoxic mechanisms,

endocrine disruption via the derailment of normal hormone signaling pathways, and outright toxicity leading to disease or death. Scientists in the USGS within DOI have established or adapted water collection, extraction, and in vitro screening assays to evaluate the bioactivity of surface water samples. These approaches circumvent the need to utilize vertebrates and minimize endpoint variability in bioactivity measures. Data from these assays are incorporated into predictive modeling analyses to identify land uses associated with predicted biological disruption. In addition, they are also applied to responsibly inform site selection for comprehensive environmental sampling. During 2018-2019 these assays were applied to augment USGS and other collaborator data sets collected from environmental surface and well waters collected in the eastern and midwestern United States.

Directive, funding to eliminate animal testing

Tags: EPA, developmental toxicity, neurotoxicity, mixtures toxicity, metrics

In a September 2019 news release, EPA announced a directive to prioritize efforts to reduce animal testing. The directive, issued by EPA Administrator Andrew Wheeler, called for reducing mammal study requests and funding 30% by 2025 and completely eliminating them by 2035. Wheeler also announced $4.25 million in funding to five universities to research the development and use of alternative test methods and strategies that reduce, refine, and/or replace vertebrate animal testing. Studies funded include in vitro models to test for developmental and reproductive toxicants, neurotoxins, and toxicity of complex environmental mixtures.

Evaluation of the avian acute oral and subacute dietary toxicity tests for pesticide registration

Tags: EPA, ecotoxicity, acute toxicity, oral toxicity, metrics

In a September 2019 news release, EPA announced a draft science policy to reduce testing of pesticides on birds when registering conventional outdoor pesticides. The draft policy describes the results and implications of a retrospective study conducted by EPA and People for the Ethical Treatment of Animals. The study explored the quantitative and qualitative contributions of risk assessment methods using single oral dose and subacute dietary toxicity endpoints to the overall conclusions of acute avian risk. The analysis indicated that, in most cases, the subacute dietary results had little impact on risk conclusions based on the use of

acute oral data alone. This finding is expected to reduce the number of animals tested by a total of 60 birds per test, for a total projected animal savings of over 700 animals per year.

Reduction of animal use through testing waivers

Tags: EPA, metrics

An article in Regulatory Toxicology and Pharmacology co-authored by ICCVAM co-chair Anna Lowit (Craig et al. 2019) summarizes the activities of EPA's Hazard and Science Policy Council (HASPOC). HASPOC was established in 2012 by the EPA Office of Pesticide Programs to consider requests for waiving animal study requirements for human health risk assessments. Since its inception, HASPOC has evaluated over 1,000 requests to waive animal studies and granted waivers in response to nearly 90% of requests. As of the article's publication in 2019, these waivers have saved over 200,000 animals, $300 million in study costs, and $6 million in study review costs.

Reassessment of dog testing requirements for food and color additive safety assessments

Tags: FDA

As FDA advances its Predictive Toxicology Roadmap, its Center for Food Safety and Applied Nutrition will reevaluate traditional approaches to toxicity testing to refine testing recommendations and include accurate predictive models of toxicity that are appropriate to use in evaluating the safe use of food additives or color additives. As part of this effort, the Center reviewed all dog studies previously submitted as part of the approval for food additive or color additive petitions. These findings may enable the Center to determine when a toxicity study in dogs could provide toxicological information not otherwise available using other experimental model systems and may help to identify endpoints for potential evaluation using modern in vitro or in silico techniques. A report on the findings of the review is in preparation.

Reduction of animal use for Leptospira vaccine potency testing

Tags: USDA, biologics, metrics

USDA is committed to reducing the use of live animals in testing and experimentation. Vaccines used in controlling animal disease frequently undergo testing in animal models to ensure they are effective. The USDA Center for Veterinary Biologics (CVB) enforces the

Virus Serum Toxin Act, which requires animal vaccines to be safe, potent, and effective. From 2013-2018, CVB developed alternatives to the codified *Leptospira* vaccine potency test to help reduce the number of hamsters used for this test. Hamsters are used because of their sensitivity to the *Leptospira* organism. Approximately 40 total hamsters per serogroup are required for potency testing each leptospiral fraction. This number encompasses 10 vaccinated animals, 10 unvaccinated control animals, and additional unvaccinated animals used to determine the LD50 of the challenge strain. A valid test that confirms sufficient vaccine potency requires 80% survival in the vaccinated animals and 80% mortality in unvaccinated control animals after administering a standardized dose of leptospiral challenge.

In order to reduce the number of animals used in this testing, CVB provided options to veterinary biologics manufacturers. An ELISA was developed by CVB to eliminate live animal potency testing after appropriate validation for a particular product line. In cases where the ELISA was not yet a reasonable option for a product line, CVB allowed back-titration hamsters to be removed from the codified test. A company that wishes to use these options must request and receive an exemption from CVB.

A separate group of hamsters is also required to propagate and maintain virulent strains for the codified test and developmental needs associated with regulated vaccines. Over 2,500 hamsters per year per facility are estimated to be used for propagation of virulent *Leptospira*. As a result, the CVB developed a cryopreservation protocol for the commonly used leptospiral strains and provides cryopreserved virulent *Leptospira* upon request.

From 2013 to 2018, the USDA Animal Care program monitored the number of hamsters listed under Category E on the annual reports from six companies that conduct *Leptospira* vaccine potency testing. Category E includes instances in which pain or distress, or potential pain or distress, is not relieved with anesthetics, analgesics, and/or tranquilizer drugs. Facilities that use animals for research, teaching, and testing are required to submit to the USDA an annual report on animal usage under the Animal Welfare Act. In 2013, the Category E designations indicated approximately 35,767 hamsters were used in total for *Leptospira* vaccine potency testing. Monitoring revealed a steady decline in animal numbers over five years such that 20,099 hamsters were used in 2018 demonstrating a 38% reduction.

CVB believes the findings indicate that these options significantly contributed to the downward trend in hamster use. The options remain available and can be found on the CVB website. At this juncture, the USDA will continue annual monitoring of hamster use in *Leptospira* vaccine potency testing and also explore other areas where the 3Rs (replacement, reduction, or refinement of animal use) can be applied.

Waiver criteria for carcinogenesis testing

Tags: EPA, carcinogenicity

For the past 40 years, questions have been raised about the relevance and regulatory utility of rodent cancer bioassays in human health risk assessment. To address these questions, EPA collaborated with experts from industry and animal welfare stakeholder groups to form the Rethinking Carcinogenicity Assessment for Agrochemicals Project. The project's goal is to determine both the appropriateness of and criteria for waiving rodent cancer bioassays for the registration of food-use pesticides. A weight-of-evidence reporting framework is being developed to determine when sufficient information is available to perform a health-protective chronic risk assessment without conducting rodent cancer bioassays. Information used includes exposure, mode-of-action, physiochemical properties, metabolism, and subchronic toxicological data from standard risk assessment endpoints. An abstract describing the framework criteria and example carcinogenicity waivers (Hilton et al.) was accepted for presentation at the 2020 SOT meeting.

Policies and Guidance for Implementation of Alternative Methods

To encourage adoption and use of NAMs, the Strategic Roadmap calls on agencies to provide clear guidance on use and acceptance of data from these approaches. During 2018 and 2019, several agencies issued guidance on the use of NAMs in the areas of testing for skin sensitization and carcinogenicity, as well as for the testing of medical devices and vaccines.

Measuring success in implementation of alternative methods and reduction of animal use

Tags: ICCVAM, metrics

In September 2019, the U.S. Government Accountability Office issued a report, "Animal Use in Research: Federal Agencies Should Assess and Report on Their Efforts to Develop and

Promote Alternatives." The report describes how the U.S. Department of Health and Human Services, USDA, and EPA ensure researchers consider the use of alternatives to animals and examines the steps the agencies have taken to facilitate the use of alternative research methods and to assess the effect of their efforts on animal use. The report recommended that ICCVAM establish a workgroup to develop metrics that ICCVAM member agencies could use to assess progress made toward reducing, refining, or replacing animal use in testing. Furthermore, the report recommended that such metrics be incorporated into ICCVAM Biennial Progress Reports. ICCVAM approved the establishment of a metrics workgroup in November 2019, and the workgroup will develop objectives and a timeline for planned activities during meetings in early 2020.

Draft science policy on non-animal methods for skin sensitization testing

Tags: EPA, skin sensitization

In an April 2018 news release, EPA announced a draft science policy to reduce animal use in testing strategies that evaluate chemicals for their ability to cause an allergic reaction, inflammation, or sensitization of the skin. The draft policy was the result of national and international collaboration among ICCVAM, NICEATM, Cosmetics Europe, the European Union Reference Laboratory for Alternatives to Animal Testing, and Health Canada's Pest Management Regulatory Agency.

Guidance on microsampling techniques in toxicokinetics studies

Tags: FDA

In May 2018, FDA announced availability of final guidance on the benefits and limitations of the use of microsampling techniques in toxicokinetics studies. Benefits of these techniques include reducing the numbers of animals needed for these studies. "S3A Guidance: Note for Guidance on Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies: Focus on Microsampling—Questions and Answers" is available on the FDA website. This international guidance was developed through FDA participation in the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH).

Guidance on nonclinical testing of cancer drugs

Tags: FDA, carcinogenicity

In June 2018, FDA released a Q&A to help stakeholders interpret international guidance on nonclinical evaluation of cancer drugs. The Q&A includes statements about reduction of animal use or use of in vitro alternatives in these studies. S9 Nonclinical Evaluation for Anticancer Pharmaceuticals – Questions and Answers – Guidance for Industry" is available on the FDA website. This international guidance was developed through FDA participation in ICH. The guidance discussed in the Q&A is being implemented by the U.S., the European Union, Japan, Brazil, Singapore, Canada, South Korea, China, Switzerland, and Taiwan, thereby increasing global impact on the 3Rs.

Guidance on nonclinical studies of microdose radiopharmaceutical diagnostic drugs
Tags: FDA

Small doses of radiolabeled drugs can be used for diagnostic purposes. Such microdoses are defined as 1/100th or less of the dose that would be expected to have a pharmacological effect.

In August 2018 FDA published final guidance on "Microdose Radiopharmaceutical Diagnostic Drugs: Nonclinical Study Recommendations" that provides recommendations for nonclinical studies of such drugs. The microdose evaluated during early clinical trials is less than or equal to 100 micrograms. Because such low doses are used, the guidance recommends an abbreviated nonclinical program. This guidance is intended to help sponsors facilitate the timely conduct of clinical trials, reduce the use of drug development resources, and reduce the use of animals by specifying study types for which in vitro methods may be used.

Guidance on nonclinical studies and labeling for oncology therapeutic radiopharmaceuticals
Tags: FDA

In August 2019 FDA published "Oncology Therapeutic Radiopharmaceuticals: Nonclinical Studies and Labeling Recommendations." This guidance provides recommendations for nonclinical studies for therapeutic radiopharmaceuticals for the treatment of cancer, and specifically describes instances where studies are not needed, potentially reducing animal use.

- When there is experience with the radionuclide or the ligand components of the radiopharmaceutical being developed, the nonclinical program can be abbreviated as needed, and the first-in-human dose can be based on clinical data as appropriate.
- In general, no toxicity studies are warranted before a first-in-human study when the radiopharmaceutical is a neat radionuclide.
- In addition, no genetic or reproductive toxicity or carcinogenicity study with the pharmaceutical, in either radiolabeled or nonradiolabeled form, is warranted during drug development or for approval.

Guidance on reproductive toxicity testing and labeling recommendations for oncology pharmaceuticals

Tags: FDA, developmental toxicity

The FDA guidance document "Oncology Pharmaceuticals: Reproductive Toxicity Testing and Labeling Recommendations" was finalized in May 2019 and provides information for the development of pharmaceuticals that are intended to treat patients with cancer. The guidance notes that, in some cases, a weight-of-evidence approach showing potential for reproductive toxicity may eliminate the need to conduct a dedicated embryofetal development study. Such weight-of-evidence approaches can consider data from alternative assays, such as fit-for-purpose in vitro, ex vivo, or nonmammalian in vivo assays. The guidance also illustrates other cases where certain developmental and reproductive toxicity testing may not be warranted.

Guidance on nonclinical development of pharmaceuticals for hematologic disorders

Tags: FDA

The FDA guidance document "Severely Debilitating or Life-Threatening Hematologic Disorders: Nonclinical Development of Pharmaceuticals" was finalized in March of 2019. This guidance is intended to streamline the development of pharmaceuticals used to treat patients with serious noncancer hematologic disorders such as sickle cell disease, hemophilia, and aplastic anemia. The guidance notes scenarios when use of animal studies can be reduced by eliminating or delaying certain nonclinical studies.

Medical Device Development Tools qualification program

Tags: FDA

The FDA Center for Devices and Radiological Health is continuing to expand acceptance of alternative information and non-animal testing to support biocompatibility evaluations of medical devices. The Center's guidance on Qualification of Medical Device Development Tools explains how new tools can be developed and qualified for a specific context of use, so that qualified tools can be used to support regulatory submissions to the Center. The policy outlined in the guidance is applicable to in vitro models to replace animal testing where appropriate. Slides, an audio presentation, and a transcript of a webinar presented to answer questions on the guidance can be found on the FDA website. To date, biocompatibility or toxicology tools have not yet been qualified under the Center's Medical Device Development Tool program. Once qualified, these tools will be published in the Medical Devices section of the FDA website.

Leadership

As an interagency committee of the U.S. Federal Government, ICCVAM takes a leadership role in promoting the use of new approach methodologies. Development of the Strategic Roadmap exemplified that leadership role. Publication of the Strategic Roadmap was accompanied or followed by the development of strategic plans by four ICCVAM member agencies to replace or reduce animal use. ICCVAM agencies also promote new testing approaches through funding test method development and collaborations with groups within and outside government.

Strategic Plans

In January 2018, ICCVAM published the Strategic Roadmap. Publication of this document was accompanied or followed by the development of strategic plans by four ICCVAM member agencies to replace or reduce animal use. These plans will support assessments of chemical safety testing needs and opportunities for new technology development.

Publication of Strategic Roadmap

Tags: ICCVAM, acute toxicity

In January 2018, ICCVAM published "A Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States." It describes a framework for safety testing that will provide more human-relevant toxicology data while reducing the use of animals. ICCVAM coordinated the development of this document during 2017 with participation from the 16 ICCVAM member agencies and multiple interagency workgroups, as well as input from a broad range of stakeholder groups. The Strategic Roadmap describes a new framework that will enable development, establish confidence in, and ensure use of new approaches to toxicity testing that improve human health relevance and reduce or eliminate the need for testing in animals. To facilitate access to the roadmap by ICCVAM's international partners, the roadmap was translated into five languages in 2018. Progress towards the roadmap goals during 2018 and 2019 focused on replacing or reducing animal use for acute toxicity endpoints. Relevant activities are described throughout this document.

Development of a strategic roadmap to promote the use of NAMs in rapid chemical assessment

Tags: DoD

DoD is developing a roadmap to promote the use of NAMs in rapid chemical hazard assessment. DoD scientists recognized the unique challenges in assessing chemical safety for diverse occupational exposure scenarios and a chemical space not well-represented outside of military interests. To address these specific concerns, DoD leadership felt that a more tailored approach was needed to fulfill the goal of reducing animal use for chemical safety testing in concert with the ICCVAM Strategic Roadmap. The DoD-specific roadmap will help laboratories across DoD to better define their chemical assessment needs and collaborate on development or refinement of appropriate non-animal approaches for testing.

Drafting of the DoD roadmap began with the "Joint DoD Technical Interchange and Roadmap Development Meeting to Promote the Use of New Approach Methodologies in Rapid Chemical Hazard Assessment." This workshop was comprised of DoD stakeholders and experts from federal and academic agencies and designed to help provide direction for future development.

While work is being led by the Army Corps of Engineers, this has been a tri-services effort with input received from all three branches of the military. Roadmap development is ongoing, with publication planned for mid-2021 following a federal stakeholder commenting period.

Strategic plan for the Toxic Substances Control Act

Tags: EPA

In June 2018, EPA published its Strategic Plan to Promote the Development and Implementation of Alternative Test Methods Within the TSCA Program. The document describes how EPA will promote development and implementation of methods and strategies that reduce, refine, or replace vertebrate animal testing to provide chemical safety information required under the Toxic Substances Control Act (TSCA). The core components of the plan are (1) identifying, developing, and integrating NAMs for TSCA decisions; (2) building confidence that the NAMs are scientific, reliable, and relevant for TSCA decisions; and (3) implementing the reliable and relevant NAMs for TSCA decisions.

The 2018 Strategic Plan established a TSCA NAM Team to take advantage of experts/resources within the Agency to implement the plan. EPA's long-term goal is to move towards making TSCA decisions with NAMs to reduce and eventually eliminate vertebrate animal testing for TSCA.

A 2016 update of TSCA required EPA to develop "a list, which the Administrator shall update on a regular basis, of particular alternative test methods or strategies the Administrator has identified that do not require new vertebrate animal testing and are scientifically reliable, relevant, and capable of providing information of equivalent or better scientific reliability and quality to that which would be obtained from vertebrate animal testing." EPA issued the original list required under TSCA in June 2018 with the Strategic Plan, and issued an update of the list in December 2019.

New strategic plan for Tox21

Tags: Tox21

Over the last 10 years, the U.S. federal Tox21 consortium has developed and evaluated in vitro HTS methods for hazard identification and mechanistic insight. Tox21 has generated data on thousands of pharmaceuticals and data-poor chemicals, characterized the limits and applications of in vitro methods, and enabled incorporation of HTS data into regulatory decisions. To more broadly address challenges in the field of toxicology, Tox21 released a new strategic and operational plan (Thomas et al. 2018) that expands the focus of its research activities. This plan proposed that Tox21 expand its portfolio of alternative test systems, address technical limitations of in vitro test systems, curate legacy in vivo toxicity testing data, establishing scientific confidence in in vitro test systems, and refine alternative methods for characterizing pharmacokinetics and in vitro assay disposition. The new Tox21 strategic and operational plan addresses key challenges to advance toxicology testing and will benefit both the organizations involved and the toxicology community.

Implementation of the FDA Predictive Toxicology Roadmap

Tags: FDA

In December 2017, FDA published its Predictive Toxicology Roadmap for integrating predictive toxicology methods into safety and risk assessments. The Predictive Toxicology

Roadmap presents a framework for new or enhanced FDA engagement in the science of toxicology that includes six elements:

1. An organizing committee to help identify areas where research is needed and reduce duplication of efforts.
2. Training in use of new test methods.
3. Communication among the agency, sponsors, and test method developers.
4. Fostering collaborations across sectors and disciplines nationally and internationally.
5. Research to identify data gaps and promote promising technologies.
6. Oversight to track progress.

FDA held a public hearing on September 12, 2018, to solicit comments on the roadmap. A public workshop on September 18, 2019, highlighted work done by FDA to support and implement the roadmap.

Funding for Alternative Methods Development

During 2018 and 2019, NIEHS and other ICCVAM member agencies supported alternative methods development through grants to small businesses and academic institutions. These grants supported development of new testing approaches for developmental toxicity, reproductive toxicity, or ecotoxicity, as well as platforms such as iPSCs, in vitro organoids, and computational approaches.

Small business grants to support alternative methods development

Tags: NIEHS, Tox21

Throughout 2018 and 2019, NIEHS provided funding for small businesses developing technologies of interest to the Tox21 program. The funding was offered as part of the 2018 and 2019 Omnibus Solicitations of NIH, Centers for Disease Control, and FDA for small business grant applications to support development and commercialization of innovative technologies. Technologies supported by NIEHS included improved or expanded testing methods for toxicity screening, computational approaches for predictive toxicology, and other technologies such as alternative or improved methods for fixing and preserving tissues.

In addition to funding offered via the Omnibus Solicitations, NIEHS offers grants to support development of specific types of technologies targeting specific endpoints. Funding offered in 2018 and 2019 supported development of:

- Technologies to assess exposure of engineered nanomaterials and characterize their effects on biological systems (July 2018).
- Engineered 3D or organotypic in vitro systems using cells from experimental animal models typically used for toxicology testing (October 2019).
- Chemical testing resources and approaches that reflect the genetic diversity among human populations, including panels of human or rodent cells or cell lines, lower organism strains with well-characterized genetic backgrounds, or in silico approaches to enhance the ability to characterize the effects of genetic variation in toxicity testing (December 2019).

Advancing toxicokinetics for efficient and robust chemical evaluations

Tags: EPA, IVIVE

Toxicokinetics describes the absorption, distribution, metabolism, and excretion of chemicals and their metabolites by the body. In July 2019, EPA requested applications for a new funding opportunity, Advancing Toxicokinetics for Efficient and Robust Chemical Evaluations. The goal of this funding was to improve the development of chemical toxicokinetic tools and approaches for broader applicability during chemical evaluations. Of particular interest were projects that would increase throughput and predictivity of current IVIVE approaches while reducing the uncertainty of these approaches. Awardees for the grant will be announced in spring 2020.

Safer chemicals research grants

Tags: EPA, developmental toxicity, ecotoxicity

EPA funds safer chemicals research grants supporting the development of innovative science to support safer, more sustainable use of chemicals in consumer products and chemicals used for other purposes such as pesticides. Using safer, more sustainable chemicals will help to better protect human and environmental health, including sensitive populations like children,

elderly, and endangered species. Much of the research funded by these grants also supports the development of alternatives to animal testing.

The following grants under this program were announced or funded during 2018 and 2019:

- Advancing Actionable Alternatives to Vertebrate Animal Testing for Chemical Safety Assessment: promoted the development and use of alternative test methods and strategies that reduce, refine, or replace vertebrate animal testing, including analog/read-across techniques, mathematical models, and tiered-testing approaches. Five grants were awarded for projects in the areas of developmental toxicity, reproductive toxicity, or ecotoxicity testing, with funding beginning in August 2019.
- Systems-Based Research for Evaluating Ecological Impacts of Manufactured Chemicals: funded development of innovative methods to better understand and predict biological and ecological consequences of exposures to manufactured chemicals in environmental systems. Three of the six projects funded by this program were completed in 2018 and 2019; the remaining three grants continue through 2020.
- Organotypic Culture Models for Predictive Toxicology: established research centers to guide the development and evaluation of organotypic culture models to accelerate translational research in predictive toxicology. Four centers were funded beginning in December 2014; funding for two centers concluded in 2019, while two centers will continue to be funded into 2020.
- New Methods in 21st Century Exposure Science: supported research to advance methods for characterizing real-world human exposure to chemicals associated with consumer products in indoor environments. Five projects were funded beginning in 2014; funding for four projects extended into 2018.

Funding for studies to improve iPSC reproducibility

Tags: NIH, stem cells

In December 2019, NIH accepted applications for Small Business Innovation Research grants to develop methods that improve the reproducibility of iPSC derivation, growth, and differentiation. Projects funded under this grant will address the significant variability

currently observed in human iPSCs in reprogramming efficiency, differentiation potential, and cell growth and stability, which is limiting the full application of these tools to research and clinical practice. Eleven NIH Institutes and centers are committing funds to this effort.

National Eye Institute 3-D Retina Organoid Challenge

Tags: NIH, stem cells

Throughout 2018 and 2019, the National Eye Institute, part of NIH, conducted the implementation phase of the 3-D Retina Organoid Challenge. This competition was designed to support development of lab-grown human retinas from stem cells. Organoids developed for the competition will mimic the structure, organization, and function of the human retina, the light-sensitive tissue in the back of the eye. The goal of these models is to advance therapies for degenerative retinal diseases such as age-related macular degeneration, glaucoma, and diabetic retinopathy. In this phase of the challenge, the National Eye Institute will award up to $100,000 each to as many as six teams whose models best address the challenge's scientific criteria. A team led by Wei Liu, Ph.D., Albert Einstein College of Medicine, received an award in December 2018; additional entries will be accepted and awards granted in fall 2020.

International Interactions

International adoption of new methods is important to advancement of NAMs within individual countries. To advance international adoption, ICCVAM and its member agencies interact frequently with international partners to facilitate harmonization and regulatory acceptance.

Contributions to OECD activities

Tags: ICCVAM, IATA, skin sensitization, eye irritation, endocrine disruptors

ICCVAM member agencies participate in the development and review of chemical testing guidelines issued by the OECD Test Guidelines Programme. OECD test guidelines are used by government, industry, and independent laboratories of the 36 OECD member countries to assess chemical safety. The U.S. National Coordinator for the OECD Test Guidelines Programme, an ex officio member of ICCVAM, solicits and collates U.S. comments on draft test guidelines and other documents of the Test Guidelines Programme. The National

Coordinator represents the United States at the annual meeting of the Working Group of National Coordinators and in other test guideline development activities. Beginning in 2018, one or more ICCVAM subject matter experts have joined the U.S. National Coordinator at this meeting.

In 2018 and 2019, ICCVAM agencies commented on draft OECD documents through the U.S. National Coordinator. ICCVAM members and/or NICEATM staff also supported the Test Guidelines Programme during 2018 and 2019 by:

- Serving on an expert group developing a guideline for defined approaches for skin sensitization. The guideline will describe the adopted defined approaches with respect to their intended regulatory purposes of hazard identification or potency subcategorization.
- Serving on expert groups for skin sensitization and eye irritation, which are tasked with reviewing guidelines and guidance documents relevant to those endpoints.
- Serving on a peer review panel for an OECD-coordinated study evaluating the use of the kinetic direct peptide reactivity assay for classifying substances for skin sensitization potency.
- Serving on the Validation Management Group – Non-animal, which focuses on evaluation of new methods for identifying endocrine disruptors.
- Helping to develop a “Case Study on the Use of an Integrated Approach to Testing and Assessment for Identifying Estrogen Receptor Active Chemicals,” submitted to the OECD Working Party on Hazard Assessment. This case study was published in September 2019, and has since been adapted to create a proposal for a defined approach for estrogenic activity, submitted to the OECD in November 2019.
- Helping to develop a "Case Study on the Use of an Integrated Approach to Testing and Assessment for Identifying Androgen Receptor Active Chemicals," submitted to the OECD Working Party on Hazard Assessment in 2019.

Participation in the International Cooperation on Alternative Test Methods

Tags: ICCVAM, skin sensitization, IATA, MPS

ICATM was created to foster dialog among national validation organizations. This dialog facilitates international cooperation in the critical areas of validation studies, independent peer review, and development of harmonized recommendations. ICATM includes member organizations from the European Union, United States, Japan, Canada, and South Korea. Brazil and China have been participating in ICATM since 2015 as observers.

ICATM Participant Organizations

- **ICCVAM** is an interagency committee of the U.S. government that coordinates technical reviews of alternative test methods and cross-agency activities relating to validation, acceptance, and harmonization of test methods. NICEATM administers ICCVAM and provides scientific support for its activities.
- **EURL ECVAM** (European Union Reference Laboratory for Alternatives to Animal Testing) is a unit within the Institute of Health and Consumer Protection in the European Union's Joint Research Centre. EURL ECVAM coordinates the validation of alternative test methods in the European Union.
- **JaCVAM** (Japanese Center for the Validation of Alternative Methods) coordinates the evaluation of alternative test methods for the Japanese National Institute of Health Sciences, its parent organization.
- **Health Canada's** Environmental Health Science and Research Bureau coordinates the evaluation of alternative test methods in Canada. The Canadian Centre for Alternatives to Animal Methods and its subsidiary, the Canadian Centre for the Validation of Alternative Methods (CaCVAM) participate as partners with Health Canada in ICATM activities.
- **KoCVAM** (Korean Center for the Validation of Alternative Methods) is part of the National Institute of Food and Drug Safety Evaluation of the South Korean Food and Drug Administration.
- The **Brazilian Center for the Validation of Alternative Methods (BraCVAM)** functions as the focal point within Brazil to identify or receive requests for test method validation. It works to implement appropriate validation studies with the

National Network of Alternative Methods to the Use of Animals within the Brazilian Ministry of Science, Technology, Innovations, and Communications.

In October 2018, representatives of NICEATM and ICCVAM attended an ICATM workshop on "Validation and Establishing Scientific Confidence" and subsequent coordination meeting. Workshop participants discussed challenges to address in evolving the process of validation, including demonstrating human health relevance without animal data, and establishing more efficient validation procedures. As a first step towards addressing the identified issues, ICATM representatives agreed that they would conduct a detailed review of OECD Guidance Document 34, the current standard for validation of chemical safety test methods. The goal of this review was to identify more specific issues to be addressed in moving forward with new approaches to validation.

ICATM observed its 10th anniversary at an October 2019 workshop. ICATM representatives reflected on progress made in the last decade toward regulatory acceptance of alternatives to animal testing, and regulatory agency representatives identified challenges and opportunities to address. Specific topics discussed during the workshop and subsequent ICATM coordination meeting included:

- Strategies to promote regulatory adoption of defined approaches to testing.
- Challenges presented in the assessment of endocrine disruptors.
- Approaches to building confidence in regulatory application of MPS.

Representatives from BraCVAM, CaCVAM, and KoCVAM attended the 2018 SACATM meeting. Representatives from EURL ECVAM, JaCVAM, KoCVAM, and CaCVAM attended the 2019 SACATM meeting.

ICCVAM member agency scientists serve on management teams or peer review panels for test method validation studies conducted by ICATM partners.

- A JaCVAM-led validation study of the amino acid derivation reactivity assay for skin sensitization potential concluded in 2018. NICEATM staff served on the validation management team, and an ICCVAM member served on the peer review panel. The

amino acid derivation reactivity assay was accepted by OECD in a 2019 revision to Test Guideline 442C.

- During 2018 and 2019, an NIEHS scientist served on the management team for a validation study led by JaCVAM. The study is evaluating the multi-immunotox assay, a screening assay that identifies potentially immunotoxic chemicals by assaying the activity of T cells and dendritic cells.

The following ICCVAM workgroups had ICATM member liaison representatives during 2018 and 2019.

ICCVAM Workgroup	ICATM Organizations with Liaison Members
Acute Toxicity Workgroup	EURL ECVAM, KoCVAM
Developmental and Reproductive Toxicity Workgroup	EURL ECVAM, JaCVAM, KoCVAM
In Vitro to In Vivo Extrapolation Workgroup	EURL ECVAM, JaCVAM
Ocular and Dermal Irritation Workgroup	EURL ECVAM, Health Canada
Read Across Workgroup	EURL ECVAM, JaCVAM
Skin Sensitization Workgroup	EURL ECVAM

Interactions with United Nations subcommittees of experts

Tags: DOT, OSHA, eye irritation, skin irritation

The Occupational Safety and Health Administration (OSHA) and the DOT Pipeline and Hazardous Materials Safety Administration participated in meetings convened by the United Nations in 2018 and 2019 to harmonize international standards on safe handling of chemicals. OSHA attended meetings of the United Nations Subcommittee of Experts on the Globally Harmonized System of Classification and Labelling of Chemicals, and DOT attended meetings of the United Nations Subcommittee of Experts on the Transport of

Dangerous Goods. Both ICCVAM agencies held public meetings to receive input from U.S. stakeholders in advance of the international meetings.

OSHA is currently leading the United States' effort to update and expand the use of non-animal test data for GHS hazard classification. The GHS chapter on skin corrosion/irritation was updated in 2019 to reflect new in vitro test methods for classifying hazards as well as updating guidance on the use of data from in silico methods. The work will continue in 2020 with an update of the eye corrosion/irritation chapter. Updates to the eye chapter will include updated and expanded guidance on the use of in vitro and in silico test data for classification as well as introducing the use of defined approaches for use of data from multiple test methods. OSHA anticipates the GHS will adopt the changes to the eye corrosion/irritation chapter by the end of 2020.

Participation on ICH

Tags: FDA, developmental toxicity, cardiotoxicity

Two FDA Centers, Drug Evaluation and Research and Biologics Evaluation and Research, pursue international harmonization of nonclinical recommendations for human pharmaceutical development through their engagement with ICH. ICH brings together regulatory authorities and the pharmaceutical industry to discuss scientific and technical aspects of drug registration. ICH's mission is to achieve greater harmonization worldwide to ensure that safe, effective, and high-quality medicines are developed and registered in the most resource-efficient manner. These activities reduce repetition of studies and reduce and refine animal use in overall drug development. New guidances recently approved will continue to contribute to the 3Rs as will future guidances that are currently being developed or revised.

FDA Q&A documents issued on ICH guidances on toxicokinetics and anticancer pharmaceuticals are discussed elsewhere in this report. The Center for Drug Evaluation and Research is engaged with other ongoing activities of ICH.

- Revision of the ICH S5 (R3) guidance on reproductive toxicology was ongoing through 2018 and 2019. The revisions propose to expand circumstances under which the outcome of "preliminary embryofetal development studies" (per ICH M3(R2))

could support clinical trials, which could lead to fewer definitive studies being conducted. In addition, the revised guidance proposes basic principles that would assist in the development and potential regulatory use of alternative assays for evaluating adverse effects on embryofetal development.

- An ICH working group is also discussing revisions to guidances related to assessing proarrythmic potential (ICH S7B and E14). The potential changes to these guidances could include incorporation of multi-ion channel assays, in silico models, in vitro human primary cell assays, and induced pluripotent cardiomyocyte assays, in addition to in vivo evaluations.

Accelerating the Pace of Chemical Risk Assessment

Tags: EPA

The advent of NAMs for generating safety information on chemicals provides an opportunity to assess what chemical risk assessment should look like in the 21st century. EPA has a leadership role in Accelerating the Pace of Chemical Risk Assessment (APCRA; Kavlock et al. 2018), an international activity designed to bring together regulators from key international regulatory agencies such as the European Chemicals Agency and Health Canada to discuss progress in applying the new tools to prioritization, screening, and application to quantitative risk assessment of differing levels of complexity. An APCRA workshop hosted by EPA in 2019 further developed the case studies initially considered at workshops in 2016 and 2017 on the development and applications of NAMs for chemical risk assessment; an online workshop is planned for early 2020. These collaborations will examine how NAMs might transform regulatory evaluation of chemicals and help to overcome barriers to acceptance by increasing confidence in the use and acceptance of NAMs in regulatory chemical risk assessment.

Collaborations

The Strategic Roadmap identified stakeholder engagement as critically important to advancing development and use of alternative methods. The articles below and others throughout this document describe collaborations to advance alternatives within agencies, among agencies, and between agencies and stakeholders.

Activities of the Tri-Services Toxicology Consortium

Tags: DoD

The mission of the Tri-Services Toxicology Consortium is to communicate, coordinate, and optimize toxicology needs throughout DoD by providing a central point of contact to DoD toxicology resources. The Consortium currently includes over 200 individuals from 11 organizations within DoD, seven of which have laboratories.

The Consortium convened three meetings during 2018 and 2019. These meetings supported ongoing efforts in improving in silico predictions of molecular targets, understanding of toxicant/microbiome interactions, and development and use of NAMs in phased approaches to toxicity data collection and hazard assessment. Significant recent accomplishments include:

- A DoD instruction that established the Consortium as a technical organization authorized and directed by the Deputy Assistant Secretary of Defense for Environment to participate in technical reviews and evaluation of techniques and methods for toxicology assessments.
- Substance-specific technical reviews concerning chromium, antimony trioxide, di(2-ethylhexyl) phthalate and hexamethylenetramine, trichloroethylene, and PFAS.
- Establishment of DoD technical representation on relevant government and scientific committees.
- Development of a draft DoD Strategic Roadmap to Promote the Use of New Approach Methodologies in Rapid Chemical Hazard Assessment.

Botanical Safety Consortium

Tags: FDA, NIEHS

In February 2019, FDA announced policies aimed at modernizing the oversight and regulation of dietary supplements. Among the steps FDA is taking to implement these policies include collaboration with industry to support the development of new products, ingredients, and delivery systems while protecting public health and safety. To support this collaboration, FDA, NIEHS, and the non-profit Health and Environmental Sciences Institute

signed a memorandum of understanding establishing the Botanical Safety Consortium. The consortium includes over 20 participants from industry, academia, and government to promote scientific advances in evaluating the safety of botanical ingredients and mixtures in dietary supplements. This group will look at novel ways to use cutting-edge toxicology tools, including alternatives to animal testing, to promote the goals of safety and effectiveness.

FDA Alternative Methods Working Group

Tags: FDA

Under the Office of the Chief Scientist in the Office of the Commissioner, FDA has chartered an Alternative Methods Working Group with representatives from all of FDA. The goals of this work group are to strengthen FDA's long commitment to promoting the development and use of new technologies and to reduce animal testing, to discuss new alternative in vitro/in silico/in vivo methods across FDA and to interact with federal government partners and other global stakeholders to facilitate discussion and development of draft performance criteria for such assays.

Rethinking Carcinogencity Assessment for Agrochemicals Project

Tags: EPA, carcinogenicity

Rodent cancer bioassays are currently required by regulatory authorities for the carcinogenicity assessment of industrial chemicals, agrochemicals, food additives, pharmaceuticals, and environmental pollutants. These studies are expensive and time-consuming and use large numbers of animals. The relevance of these studies to human biology has also been questioned. For this reason, there is increased interest in mechanistic approaches for carcinogenicity assessment that reduce testing on animals and provide information more relevant to protecting human health.

EPA is partnering with agrochemical industry stakeholders, the University of South Florida, and the PETA International Science Consortium Ltd. in the Rethinking Carcinogenicity Assessment for Agrochemicals Project. This project is exploring how requirements for rodent cancer bioassays might be replaced with a weight-of-evidence approach that would consider criteria based on potential triggers and indicators of carcinogenesis. In this project, case studies will be reviewed to determine whether the cancer risk indicated in bioassay results

could have been predicted by weight-of-evidence criteria. The goal of the project is to produce a guidance document that will describe a weight-of-evidence framework for carcinogenicity assessment. The project will be described in an SOT webinar to be presented in early 2020 and in two abstracts (Papieni; Hilton et al.) accepted for presentation at the SOT 2020 annual meeting.

Other EPA collaborations with stakeholders

Tags: EPA, inhalation toxicity, acute toxicity, oral toxicity

EPA has formed strategic partnerships with organizations in multiple sectors to encourage the use of alternative toxicity testing methods in decision making. These activities will lead to more timely chemical evaluations that may better inform protection of human health and the environment.

- EPA is collaborating with NICEATM and international partners on an analysis of dermal absorption data to assess the feasibility of using data from in vitro studies alone to estimate dermal absorption.
- EPA is collaborating with NICEATM, the PETA International Science Consortium Ltd., and CropLife America member companies to develop an in vitro defined approach for hazard classification of eye irritation potential of agrochemical formulations.
- EPA collaborated with Syngenta to support development of a 3D in vitro inhalation toxicity model to derive a point of departure for inhalation toxicity for the fungicide chlorothalonil.
- EPA is working with Unilever to advance chemical safety for consumer products through case studies focused on five chemicals. The goal of the collaboration is to develop new, more efficient approaches for chemical hazard assessment, including high-throughput transcriptomics. The case studies are using new chemical data, such as data from ToxCast, to evaluate chemicals.
- EPA and Proctor & Gamble are collaborating to advance the use of a new technology developed by EPA researchers that incorporates metabolic competency into HTS assays.

- EPA is collaborating with the Minnesota Department of Health to use alternative approaches to evaluate chemicals in drinking water.
- A retrospective study conducted by EPA and People for the Ethical Treatment of Animals explored the quantitative and qualitative contributions of risk assessment methods using single oral dose and subacute dietary toxicity endpoints to the overall conclusions of acute avian risk.

Details of these and other projects can be found on EPA's Collaborative Agreements for Computational Toxicology Research webpage.

About ICCVAM and NICEATM

ICCVAM is supported by the National Toxicology Program Interagency Center for the Evaluation of Alternative Methods. The two groups work collaboratively to evaluate new and improved testing approaches applicable to the needs of U.S. federal agencies. The articles below provide background information on ICCVAM and NICEATM.

ICCVAM Establishment and Purpose

U.S. regulatory agencies are charged to protect human and animal health and the environment. To do this, agencies must determine the hazards presented by substances such as pesticides, consumer products, and workplace chemicals. Testing these substances provides information about possible hazards and enables informed decisions about responsible use, storage, and disposal.

Many currently accepted test methods use laboratory animals. Alternative test methods are methods that replace animal use with non-animal test systems or lower species, reduce the number of animals required for a specific test procedure, or refine animal use to enhance animal well-being and lessen or avoid pain and distress. Collectively, the principles of replacement, reduction, or refinement of animal use for scientific research or product safety testing are referred to as the 3Rs. More recently, the term "new approach methodologies" has been adopted as a broadly descriptive reference to any non-animal technology, methodology, approach, or combination of these that can be used to provide information on chemical hazard and risk assessment.

The ICCVAM Authorization Act of 2000 (42 U.S.C. 285*l*-3) established the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) to

> "establish, wherever feasible, guidelines, recommendations, and regulations that promote the regulatory acceptance of new or revised scientifically valid safety testing methods that protect human and animal health and the environment while reducing, refining, and replacing animal tests and ensuring human safety and product effectiveness."

The ICCVAM Authorization Act states that the purposes of ICCVAM are to:

- Increase the efficiency and effectiveness of federal agency test method review.
- Eliminate unnecessary duplicative efforts and share experiences between federal regulatory agencies.
- Optimize utilization of scientific expertise outside the federal government.
- Ensure that new and revised test methods are validated to meet the needs of federal agencies.
- Reduce, refine, and replace the use of animals in testing, where feasible.

ICCVAM is a permanent interagency committee of NIEHS under the NTP Interagency Center for the Evaluation of Alternative Methods (NICEATM).

ICCVAM Member Agencies

- Agency for Toxic Substances and Disease Registry (ATSDR)
- National Cancer Institute (NCI)
- National Institute for Occupational Safety and Health (NIOSH)
- National Institute of Environmental Health Sciences (NIEHS)
- National Institute of Standards and Technology (NIST)
- National Institutes of Health (NIH)
- National Library of Medicine (NLM)
- Occupational Safety and Health Administration (OSHA)
- U.S. Consumer Product Safety Commission (CPSC)
- U.S. Department of Agriculture (USDA)
- U.S. Department of Defense (DoD)
- U.S. Department of Energy (DOE)
- U.S. Department of the Interior (DOI)
- U.S. Department of Transportation (DOT)
- U.S. Environmental Protection Agency (EPA)
- U.S. Food and Drug Administration (FDA)

ICCVAM Duties and Activities

The ICCVAM Authorization Act directs ICCVAM to carry out the following duties:

- Coordinate the technical review and evaluation of new, revised, or alternative test methods.
- Foster interagency and international harmonization of test protocols that encourage replacing, reducing, and refining animal test methods.
- Assist with and provide guidance on validation criteria and processes.
- Promote the acceptance of scientifically valid test methods.
- Promote awareness of accepted test methods.
- Submit ICCVAM test method recommendations to appropriate U.S. federal agencies.
- Consider requests from the public to review and evaluate new, revised, or alternative test methods that have evidence of scientific validity.
- Make ICCVAM's final test recommendations available to the public.
- Prepare reports on ICCVAM progress and accomplishments under the Act and make them available to the public.

How NICEATM Supports ICCVAM

NICEATM, an office within the NIEHS Division of NTP, provides technical and scientific support for ICCVAM and ICCVAM working group activities, peer review panels, expert panels, workshops, and validation efforts.

In addition to providing support for ICCVAM, NICEATM:

- Supports NTP activities, especially those contributing to the U.S. government's interagency Tox21 initiative.
- Conducts analyses and evaluations, and coordinates independent validation studies on novel and high-priority alternative testing approaches.
- Provides information to test method developers, regulators, and regulated industry through its website and workshops on topics of interest.

NICEATM staff

NIEHS

Warren Casey, Ph.D., DABT, Senior Toxicologist; Director

Nicole Kleinstreuer, Ph.D., Deputy Director

Elizabeth Maull, Ph.D., Toxicologist

Matthew Stout, Ph.D., Toxicologist, Project Officer

NICEATM Contract Staff (Integrated Laboratory Systems, Inc.)

David Allen, Ph.D., Principal Investigator

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Jaleh Abedini, M.S.

Shannon Bell, Ph.D.

Patricia Ceger, M.S., DABT

Xiaoqing Chang, Ph.D., DABT

Neepa Choksi, Ph.D.

Amber Daniel, M.S.

Jon Hamm, Ph.D.

Agnes Karmaus, Ph.D.

Isabel Lea, Ph.D.

Kamel Mansouri, Ph.D.

Jason Phillips (subcontractor, Sciome LLC)

John Rooney, Ph.D.

Catherine Sprankle, M.S.

Judy Strickland, Ph.D., DABT

James Truax, M.S.

ICCVAM Advisory Committee

The ICCVAM Authorization Act established the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM). SACATM advises the Director of NIEHS, NICEATM, and ICCVAM about NICEATM and ICCVAM activities.

SACATM, which is directed by its charter to meet at least once each fiscal year, met in September 2018 and September 2019.

Roster of SACATM Members 2018-2019

Name	Title	Company	Appointment End Year
Michael B. Bolger, Ph.D.	Chief Scientist	Simulations Plus, Inc., Lancaster, CA	2020
Joseph L. Charest, Ph.D.	Biomedical Solutions Program Manager	The Charles Stark Draper Laboratory, Inc., Cambridge, MA	2022
Amy Clippinger, Ph.D.	Director	PETA International Science Consortium Ltd., Washington, DC	2022
Kelly P. Coleman, Ph.D., DABT, RAC	Distinguished Scientist and Technical Fellow	Medtronic PLC, Minneapolis, MN	2020
K. Nadira De Abrew, Ph.D.	Senior Scientist (Toxicologist)	The Procter & Gamble Company, Cincinnati, OH	2022
Sean C. Gehen, Ph.D., DABT	Regulatory Sciences Team Leader	Corteva Agriscience, Indianapolis, IN	2022
Hisham K. Hamadeh, Ph.D., DABT, M.B.A.	Vice President, Global Head of Data Sciences	Genmab US, Inc., Princeton, NJ	2019
William P. Janzen	Executive Director of Lead Discovery	Epizyme, Inc., Cambridge, MA	2018
Lawrence Milchak, Ph.D., DABT	Senior Manager, Toxicology and Strategic Services	3M Corporation, St. Paul, MN	2019
Pamela Spencer, Ph.D., DABT	Director of Regulatory and Product Stewardship	ANGUS Chemical Company, Buffalo Grove, IL	2019
ClarLynda Williams-Devane, Ph.D.	Associate Professor of Mathematics and Computer Science; Discipline Coordinator of Bioinformatics	Fisk University Nashville, TN	2020

Wei Xu, Ph.D.	Associate Professor, Department of Oncology	McArdle Laboratory for Cancer Research, University of Wisconsin at Madison, Madison, WI	2018
Hao Zhu, Ph.D.	Assistant Professor, Department of Chemistry	Rutgers University, Camden, NJ	2019

Reference Pages

Agency Representatives in 2018 and 2019

The individuals listed on this page served as representatives from ICCVAM member agencies in 2018 and 2019. ICCVAM includes three types of representatives:

- The **Principal Agency Representative** is the primary source of communication from his or her agency to the committee and NICEATM. He or she regularly attends all ICCVAM meetings and teleconferences, coordinates participation of other representatives from the agency, and casts the agency's vote on occasions when formal voting is required.
- **Alternate Agency Representatives** participate in ICCVAM committee activities in place of the Principal when necessary.
- An agency may designate one or more **Other Agency Representatives** to participate in ICCVAM to provide broader representation or expertise.

Agency (Office)	Representative	Representative Type (as of December 2019)	Service Continuing Into 2020
Agency for Toxic Substances and Disease Registry	Moiz Mumtaz, Ph.D.	Principal	Yes
Agency for Toxic Substances and Disease Registry	Edward Murray, Ph.D.	Other	Yes
National Cancer Institute	Mark Miller, Ph.D., FAC-COR III	Principal	Yes
National Cancer Institute	Ron Johnson, Ph.D.	Alternate	Yes
National Institute for Occupational Safety and Health	Richard Probst, D.V.M., M.P.H., DACLAM	Principal	Yes
National Institute for Occupational Safety and Health	Karen Heller Taylor, D.V.M.	Principal	

Agency (Office)	**Representative**	**Representative Type (as of December 2019)**	**Service Continuing Into 2020**
National Institute of Environmental Health Sciences	Brian Berridge, D.V.M., Ph.D., DACVP	Principal	Yes
National Institute of Environmental Health Sciences	Daniel Shaughnessy, Ph.D., DABT	Principal	
National Institute of Environmental Health Sciences	Richard Paules, Ph.D.	Alternate	
National Institute of Environmental Health Sciences	Warren Casey, Ph.D., DABT	Other	Yes
National Institute of Environmental Health Sciences	Nicole Kleinstreuer, Ph.D.	Other	Yes
National Institute of Environmental Health Sciences	Elizabeth Maull, Ph.D.	Other	Yes
National Institute of Standards and Technology	John Elliott, Ph.D.	Principal	Yes
National Institute of Standards and Technology	Elijah Petersen, Ph.D.	Alternate	Yes
National Institutes of Health	Seila Selimovic, Ph.D.	Principal	
National Institutes of Health	Christine Kelley, Ph.D.	Principal	
National Institutes of Health	Harold Watson, Ph.D.	Alternate	Yes
National Library of Medicine	Pertti (Bert) Hakkinen, Ph.D.	Principal	Yes
National Library of Medicine	George Fonger	Alternate	
National Library of Medicine	Jeanne Goshorn, M.S.	Alternate	Yes
Occupational Safety and Health Administration	Surender Ahir, Ph.D.	Principal	Yes

Agency (Office)	**Representative**	**Representative Type (as of December 2019)**	**Service Continuing Into 2020**
Occupational Safety and Health Administration	Deana Holmes, M.T.	Alternate	Yes
U.S. Consumer Product Safety Commission	John Gordon, Ph.D.	Principal	Yes
U.S. Consumer Product Safety Commission	Kristina Hatlelid, Ph.D.	Alternate	Yes
U.S. Consumer Product Safety Commission	Eric Hooker, M.S.	Alternate	Yes
U.S. Consumer Product Safety Commission	Joanna Matheson, Ph.D.	Alternate	Yes
U.S. Department of Agriculture	Carol Clarke, D.V.M., DACLAM	Principal	Yes
U.S. Department of Agriculture	Kristina Adams, M.S.	Alternate	Yes
U.S. Department of Agriculture	Katherine Horak, Ph.D.	Other	Yes
U.S. Department of Agriculture	Patrice Klein, M.S., V.M.D., DACPV, DACVPM	Other	Yes
U.S. Department of Defense	Marla Brunell, D.V.M.., M.P.H., DACLAM, DACVPM	Alternate	
U.S. Department of Defense	Matthew Johnson, D.V.M., DACLAM	Alternate	Yes
U.S. Department of Defense	Emily N. Reinke, Ph.D., DABT (Co-chair)	Alternate (acting Principal)	Yes
U.S. Department of Energy	Prem C. Srivastava, Ph.D.	Principal	Yes
U.S. Department of the Interior	Barnett A. Rattner, Ph.D.	Principal	Yes

Agency (Office)	**Representative**	**Representative Type (as of December 2019)**	**Service Continuing Into 2020**
U.S. Department of the Interior	Jessica Leet, Ph.D.	Alternate	Yes
U.S. Department of the Interior	Tim Bargar, Ph.D.	Other	Yes
U.S. Department of the Interior	Adria Elskus, Ph.D.	Other	Yes
U.S. Department of the Interior	Paula F.P. Henry, Ph.D.	Other	Yes
U.S. Department of the Interior	Luke R. Iwanowicz, Ph.D.	Other	Yes
U.S. Department of Transportation	Steve Hwang, Ph.D.	Principal	Yes
U.S. Department of Transportation	Ryan Vierling, Ph.D.	Alternate	Yes
U.S. Environmental Protection Agency (Office of Pesticide Programs)	Anna Lowit, Ph.D. (Co-chair)	Principal	Yes
U.S. Environmental Protection Agency (Office of Pesticide Programs)	Monique Perron, Ph.D.	Alternate	
U.S. Environmental Protection Agency (Office of Pollution Prevention and Toxics)	Louis (Gino) Scarano, Ph.D.	Other	Yes
U.S. Environmental Protection Agency (Office of Research and Development)	Pamela Noyes, Ph.D.	Alternate	Yes
U.S. Environmental Protection Agency (Office of Research and Development)	Stephanie Padilla, Ph.D.	Alternate	Yes
U.S. Food and Drug Administration (Center for Biologics Evaluation and Research)	Robin Levis, Ph.D.	Other	Yes

Agency (Office)	**Representative**	**Representative Type (as of December 2019)**	**Service Continuing Into 2020**
U.S. Food and Drug Administration (Center for Biologics Evaluation and Research)	Allen Wensky, Ph.D.	Other	Yes
U.S. Food and Drug Administration (Center for Devices and Radiological Health)	Simona Bancos, Ph.D.	Other	Yes
U.S. Food and Drug Administration (Center for Devices and Radiological Health)	Jennifer Goode	Alternate	Yes
U.S. Food and Drug Administration (Center for Devices and Radiological Health)	Rakhi Dalal-Panguluri, Ph.D.	Other	Yes
U.S. Food and Drug Administration (Center for Drug Evaluation and Research)	Paul C. Brown, Ph.D.	Other	Yes
U.S. Food and Drug Administration (Center for Drug Evaluation and Research)	Jill Merrill, Ph.D.	Other	Yes
U.S. Food and Drug Administration (Center for Food Safety and Applied Nutrition)	Patrick Crittenden, Ph.D.	Other	Yes
U.S. Food and Drug Administration (Center for Food Safety and Applied Nutrition)	Suzanne Fitzpatrick, Ph.D., DABT	Principal	Yes
U.S. Food and Drug Administration (Center for Food Safety and Applied Nutrition)	Geoffrey Patton, Ph.D.	Other	

Agency (Office)	**Representative**	**Representative Type (as of December 2019)**	**Service Continuing Into 2020**
U.S. Food and Drug Administration (Center for Food Safety and Applied Nutrition)	Nakissa Sadrieh, Ph.D.	Other	
U.S. Food and Drug Administration (Center for Tobacco Products)	Pei-Hsuan (Chris) Hung, Ph.D.	Other	
U.S. Food and Drug Administration (Center for Tobacco Products)	Jueichuan (Connie) Kang, Ph.D.	Other	Yes
U.S. Food and Drug Administration (Center for Tobacco Products)	Arianne Motter, Ph.D.	Other	
U.S. Food and Drug Administration (Center for Veterinary Medicine)	M. Cecilia Aguila, D.V.M.	Other	Yes
U.S. Food and Drug Administration (Center for Veterinary Medicine)	Li You, Ph.D.	Other	Yes
U.S. Food and Drug Administration (National Center for Toxicological Research)	Donna Mendrick, Ph.D.	Other	Yes
U.S. Food and Drug Administration (National Center for Toxicological Research)	Mugimane (Manju) Manjanatha, Ph.D.	Other	Yes
U.S. Food and Drug Administration (Office of the Chief Scientist)	Tracy Chen, Ph.D., DABT	Other	Yes
U.S. National Coordinator for OECD Test Guidelines Programme	Wanda Hall	Other	Yes

NICEATM and ICCVAM Publications, 2018-2019

Landing page text: This page lists publications issued in 2018 and 2019 that describe NICEATM and ICCVAM activities.

NICEATM and ICCVAM Reports

ICCVAM. 2018. A Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States [Internet]. Research Triangle Park (NC): National Institute of Environmental Health Sciences [cited 09 March 2020]. Available: https://ntp.niehs.nih.gov/go/iccvam-rdmp. https://dx.doi.org/10.22427/NTP-ICCVAM-ROADMAP2018.

ICCVAM. 2018. Biennial Progress Report 2016-2017: Interagency Coordinating Committee on the Validation of Alternative Methods [Internet]. Research Triangle Park (NC): National Institute of Environmental Health Sciences [cited 09 March 2020]. Available: https://ntp.niehs.nih.gov/iccvamreport/2017/index.html.

Federal Register Notices

All *Federal Register* notices issued by NICEATM can be found on the NTP website at http://ntp.niehs.nih.gov/go/frn.

NIEHS. 2018. ICCVAM Communities of Practice Webinar on Machine Learning in Toxicology: Fundamentals of Application and Interpretation; notice of public webinar; registration information. Federal Register. 83(5):823-824.

NIEHS. 2018. A Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States; availability of report. Federal Register. 83(35):7487.

NIEHS. 2018. Predictive Models for Acute Oral Systemic Toxicity; notice of meeting; registration information. Federal Register. 83(36):7750-7751.

NIEHS. 2018. ICCVAM notice of public meeting; request for public input. Federal Register. 83(67):14867-14869.

NIEHS. 2018. Request for data and information on technologies used for identifying potential developmental toxicants. Federal Register. 83(88):20082.

NIEHS. 2018. Request for data and information on technologies used to detect and measure botulinum neurotoxin. Federal Register. 83(114):27622-27623.

NIEHS. 2018. ICCVAM Biennial Progress Report: 2016-2017; availability of report. Federal Register. 83(152):38717-38718.

NIEHS. 2019. ICCVAM notice of public meeting; request for public input. Federal Register. 84(71):14953-14954.

NIEHS. 2019. ICCVAM Communities of Practice Webinar on Use of Animal-free Affinity Reagents. Federal Register. 84(228):61567-61568.

Journal Articles

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Glossary of Key Terms

3Rs: the principles of replacement, reduction, or refinement of animal use for scientific research or product safety testing.

Accuracy: the closeness of agreement between a test method result and an accepted reference value, or the test method's proportion of correct outcomes.

Acute systemic toxicity: the immediate or near-immediate effect of a toxic substance after it is absorbed and distributed throughout the body. Different acute systemic toxicities are distinguished by the route of exposure: by ingestion (oral), through the skin (dermal), or by inhalation.

Adverse outcome pathway (AOP): a conceptual framework constructed from existing knowledge that relates exposure of a type of toxic substance to subsequent steps that result in illness or injury.

Aerosolized particulates: fine solid or liquid particles, generally 10 microns or less in size, suspended in a gas.

Agonist: a substance that increases activity of the target receptor.

Algorithm: a set of steps that are followed to complete a computational process.

Allergic contact dermatitis: an allergic reaction that results from repeated direct skin contact with a skin sensitizer. Clinical signs of allergic contact dermatitis include redness, swelling, blistering, and itching.

Alternative methods: testing methods or approaches that replace, reduce, or refine animal use; the term new approach methodologies is also used and is becoming more prevalent.

Androgen: a class of hormones, produced largely by the testes, that serve as the primary male hormones.

Androgen receptor: a protein molecule to which an androgen or androgen-like substance can attach. This interaction produces a chemical signal or triggers a cellular response.

Antagonist: a substance that decreases activity of the target receptor.

Applicability domain: a range of chemicals and properties for which a test method has been proven useful.

Bioactivity: the manner in which a chemical affects or interacts with living tissue.

Bioavailability: potential for chemical absorption and distribution throughout the body and into cells, or the extent of chemical accessibility at a physiologically active site.

Biomimetic: referring to a product or material produced by artificial mechanisms that mimics a natural product or material.

Cardiomyocytes: heart cells.

Cardiotoxicity: toxicity to the heart.

Chorion: in fish, the outermost membrane of an egg.

Countermeasure drugs: drugs developed to prevent or treat harm from a biological, chemical, radiological, or nuclear agent.

Cytotoxic: the ability of a substance to kill or harm cells.

Defined approach: a testing strategy that consists of input data generated from identified methods and a data interpretation procedure, such as a machine learning model, flowchart, or decision tree, through which the data are evaluated.

Developmental toxicity: effects observed in offspring that occur as a result of chemical exposures of the pregnant mother.

Dosimetry: measurement or calculation of a dose of substance delivered to a target tissue.

Ecotoxicity testing: refers both to the assessment of chemical effects on invertebrates, fish, birds, plants, and other wild organisms and testing of soil, sediment, or effluents for the presence of toxic compounds.

Endocrine disruptor: a natural or man-made substance that may interfere with the endocrine system and produce adverse health effects.

Endothelium: the tissue that forms a single layer of cells lining various organs and cavities of the body.

Environmental fate parameters: properties of a chemical, such as biodegradability and soil adsorption, that affect how it will behave if released into the environment.

Estrogen: a class of hormones, produced largely by the ovaries, that serve as the primary female hormones.

Estrogen receptor: a protein molecule to which an estrogen or estrogen-like substance can attach. This interaction produces a chemical signal or triggers a cellular response.

Ex vivo: refers to an assay using tissue that has been removed from a multicellular organism and conducted while the tissue is still viable.

Formulation: a mixture of chemicals prepared according to a specific procedure to ensure a desired effect when used, improve handling properties, or achieve other desired product goals.

G protein-coupled receptor: receptors located in the cell membrane involved in cell signaling. G protein-coupled receptors are important drug target and involved in many diseases.

Genotoxic: capable of damaging DNA.

Genotype: the genetic makeup of an individual organism.

Harmonization: the act of making systems or laws similar among different companies, countries, etc., so the organizations using those systems or laws can operate more easily within the different venues.

Hazard classification: assignment of a substance to a category according to results of toxicity testing, most often for labeling purposes.

Hepatic: referring to the liver.

Hepatocyte: the main functional cell of the liver.

Hepatotoxicity: toxicity to the liver.

High-content screening: an approach that uses fluorescent tagging and automated imaging to assess changes in the structure and composition of individual cells in a high-throughput manner.

High-throughput screening (HTS): a testing approach that uses robotics, liquid-handling devices, detectors, and associated software to quickly conduct a large number of chemical or biochemical tests.

Homeostasis: the maintenance of physical and chemical conditions by living systems to allow optimal functioning of the organism**.**

In silico: refers to analyses that are carried out on a computer or via computer simulation.

In vitro: refers to assays that are carried out in an artificial system, such as a test tube or assay plate, using small single-cell or multicellular organisms, cultured cells, or cellular components.

In vitro to in vivo extrapolation (IVIVE): an analysis conducted to relate the test chemical concentration causing a response in an in vitro system to concentrations that result in human or animal (in vivo) illness or injury at the target tissue.

In vivo: refers to assays carried out using multicellular organisms, typically rodents or other mammals.

Induced pluripotent stem cells (iPSCs): stem cells derived from mature non-sex cells that have the potential to differentiate into various types of cells.

Integrated approach to testing and assessment (IATA): an approach that considers all available relevant information about a substance in a weight-of-evidence assessment to inform a regulatory decision regarding hazard or risk, or to indicate that specific additional tests are needed.

LC50: in traditional animal tests for acute inhalation or aquatic toxicity, the concentration that causes death in 50 percent of the animals tested; a value used to categorize toxic substances and determine the hazard phrases used on product labels.

LD50: in traditional animal tests for acute systemic oral or dermal toxicity, the dose that causes death in 50 percent of the animals tested; a value used to categorize toxic substances and determine the hazard phrases used on product labels.

Leukocyte: any of the colorless blood cells of the immune system; see also lymphocytes.

Lympocytes: a type of immune cell that is made in the bone marrow and is found in the blood and in lymph tissue.

Machine learning: the study and construction of computer algorithms that, once trained on a set of data, can make predictions or decisions about a different set of data.

Metabolism: the sum of the processes by which a particular substance is handled in a living organism, such as assimilation and incorporation or detoxification and excretion.

Microbiome: the microorganisms in a particular environment, including the body or part of a body.

Microfluidic technology: systems used to process or manipulate small volumes of fluids (nanoliters or less).

Microphysiological systems (MPS): in vitro models of organs composed of cells and structural materials that are designed to reproduce the function of living organs; also referred to as organs-on-a-chip or tissue chips.

Microsampling: extraction of blood, plasma, or serum from experimental animals in quantities of 50 microliters or less; microsampling is generally less stressful for an animal and may allow reduction of animal use.

Microtiter plate: a flat plate with multiple wells used as small test tubes.

Nanomaterial: a substance made up of particles that measure no more than 100 nanometers in at least one dimension.

Neurotoxicity: toxicity to the brain or other parts of the nervous system.

New approach methodologies (NAMs): testing methods or approaches that replace, reduce, or refine animal use; the term alternative methods has also been widely used.

Nonclinical studies: tests of candidate drugs conducted in animals or in vitro assays, typically before testing in humans to assess toxicity and determine safe doses; the term preclinical studies is also used.

Ontologies: standardized nomenclature systems**.**

Pharmacokinetics: an evaluation of the rate at which a chemical is absorbed, distributed, metabolized, and excreted once it enters the body, as a means to determine the relationship between exposure and toxicity (see also toxicokinetics).

Pharmacokinetic (PK) model: a mathematical model created to describe the process of absorption, distribution, metabolism, and excretion of a chemical through the body. One-compartment models treat all organs as a single unit, whereas physiologically based models are usually multicompartment models with separate compartments corresponding to individual or combined organs that are interconnected by blood flows.

Phenotype: observable characteristics of an organism resulting from the interaction of its genetic makeup with the environment.

Phenotyping: collection of information on the characteristics of an organism.

Physicochemical properties: referring to the physical or chemical properties of a substance.

Point of departure: the lowest dose or concentration at which a treatment-related response is observed.

Proarrythmic: refers to a drug that causes problems with heart rhythm, or worsens existing problems.

Quantitative structure-activity relationship (QSAR) models: classification models that predict the activities of chemicals with unknown properties by relating them to properties of known chemicals.

Read-across: a computational technique that uses toxicity data from one or more known (source) chemicals to predict toxicity for another (target) chemical, usually but not always on the basis of structural similarity.

Reference chemical: a chemical that causes a specific well-characterized biological effect, and therefore, can be used to assess the performance of a test method designed to measure that effect.

Reference data: data from an accepted test method that can be used to assess the performance of a new test method designed to measure an analogous effect.

Refinement alternative: a test method that modifies procedures to enhance animal well-being, and lessen or avoid pain and distress in animals.

Relevance: the extent to which a test method accurately measures a biological effect of interest in a species of interest.

Reliability: the extent to which a test method provides reproducible results over time and in different laboratories.

Replacement alternative: a test method that replaces animals with a non-animal system or one animal species with a phylogenetically lower one.

Reproductive toxicity: chemical effects on the reproductive system that interfere with an organism's sexual function or fertility.

Risk assessment: the process of characterizing the potential risk posed by a chemical, taking into consideration the hazards posed by the chemical, the dose of the chemical needed to cause health problems, and the probability of exposure at that dose.

Serogroup: a group of variants within a species of virus or bacteria having common cell surface antigens.

Single nucleotide polymorphisms (SNPs): differences in a single nucleotide within an organism's DNA. While most SNPs have no effect on health or development, some of these genetic differences can either have functional effects or serve as markers for disease susceptibility.

Six-pack studies: acute toxicity tests that generate data required by the EPA for pesticide registration. They include tests for acute systemic toxicity by the oral, dermal, and inhalation routes; skin and eye irritation; and skin sensitization.

Skin sensitization: a hypersensitivity reaction that occurs when a susceptible person comes in direct skin contact with an allergen, termed a skin sensitizer.

Skin sensitization potency: the relative amount of a substance that produces a skin sensitization reaction.

Steatosis: accumulation of fat droplets composed mostly of triglycerides within liver cells, which can be a sign of toxicity from alcohol or other chemicals.

Stem cells: undifferentiated cells of a multicellular organism that can produce indefinitely more cells of the same type and can also be induced to differentiate into other types of cells.

Steroidogenesis: the biological process responsible for synthesis of steroid hormones from cholesterol.

Subacute: Animal experiment designed to study effects produced by the test substance when administered either in repeated doses or continually in food, drinking-water, or air over a period of between 24 h and 28 days.

Subchronic: Animal experiment designed to study effects produced by the test substance when administered either in repeated doses or continually in food, drinking-water, or air over a period of up to about 90 days.

Sublethal: a dose or concentration of a substance that is not high enough to cause death.

Titration (virology): inoculation of an animal with a virus preparation to assess the potency of the preparation for use in vaccine testing.

Toxicant: a toxic or poisonous substance.

Toxicokinetics: an evaluation of the rate at which a chemical is absorbed, distributed, metabolized, and excreted once it enters the body, as a means to determine the relationship between exposure and toxicity (see also pharmacokinetics).

Transactivation assay: an in vitro assay using cells containing a DNA plasmid that includes a regulatory sequence positioned upstream of the coding sequence of a reporter protein. Production of protein is proportional to stimulation of the regulatory sequence by a treatment chemical, and is often measured using light or color.

Transcriptomics: the analysis of overall gene expression in a cell or tissue to assess cell function or response to toxicity.

Uterotrophic assay: an assay conducted in female rodents that measures the estrogenic activity of a chemical by assessing the chemical's effect on the weight of the uterus.

Validation: a process by which the reliability and relevance of a test method are established for its intended application.

Viability: ability to live, especially under specific conditions.

Abbreviations and Acronyms

3Rs	Principles of replacement, reduction, or refinement of animal use for scientific research or product safety testing
AFRL	Air Force Research Laboratory (U.S. Department of Defense)
AOP	Adverse outcome pathway
APCRA	Accelerating the Pace of Chemical Risk Assessment
ATSDR	Agency for Toxic Substances and Disease Registry
AWIC	Animal Welfare Information Center (U.S. Department of Agriculture)
BoNT	Botulinum neurotoxin
BraCVAM	Brazilian Center for the Validation of Alternative Methods
CaCVAM	Canadian Centre for the Validation of Alternative Methods
CATMoS	Collaborative Acute Toxicity Modeling Suite
CCDC CBC	U.S. Army Combat Capabilities Development Command Chemical Biological Center (U.S. Department of Defense)
CERAPP	Collaborative Estrogen Receptor Activity Prediction Project
CoMPARA	Collaborative Modeling Project for Androgen Receptor Activity
CPSC	U.S. Consumer Product Safety Commission
CVB	Center for Veterinary Biologics (U.S. Department of Agriculture)
DoD	U.S. Department of Defense
DOE	U.S. Department of Energy
DOI	U.S. Department of the Interior
DOT	U.S. Department of Transportation
DPRA	Direct peptide reactivity assay
DSStox	Distributed Structure-Searchable Toxicity (U.S. Environmental Protection Agency)
ELISA	Enzyme linked immunosorbent assay
EPA	U.S. Environmental Protection Agency
EURL ECVAM	European Union Reference Laboratory for Alternatives to Animal Testing
FDA	U.S. Food and Drug Administration
FXRα	Farnesoid X receptor alpha

GHS	United Nations Globally Harmonized System of Classification and Labeling of Chemicals
HASPOC	Hazard and Science Policy Council (U.S. Environmental Protection Agency)
h-CLAT	Human cell line activation test
HTS	High-throughput screening
httk	High-throughput toxicokinetic (software package, U.S. Environmental Protection Agency)
IATA	Integrated approach to testing and assessment
ICATM	International Cooperation on Alternative Test Methods
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ICE	Integrated Chemical Environment (National Toxicology Program)
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ILS	Integrated Laboratory Systems, Inc.
iPSC	Induced pluripotent stem cell
IVIVE	In vitro to in vivo extrapolation]
JaCVAM	Japanese Center for the Validation of Alternative Methods
KoCVAM	Korean Center for the Validation of Alternative Methods
LC50	In traditional acute inhalation or aquatic toxicity tests, the concentration that produces lethality in 50% of the animals tested
LD50	In traditional acute dermal or oral systemic toxicity tests, the dose that produces lethality in 50% of the animals tested
MPS	Microphysiological systems
NAM	New approach methodology
NCATS	National Center for Advancing Translational Sciences (National Institutes of Health)
NCCT	National Center for Computational Toxicology (U.S. Environmental Protection Agency)
NCI	National Cancer Institute (National Institutes of Health)
NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods

NIEHS	National Institute of Environmental Health Sciences (National Institutes of Health)
NIH	National Institutes of Health
NIOSH	National Institute for Occupational Safety and Health
NIST	National Institute of Standards and Technology
NLM	National Library of Medicine (National Institutes of Health)
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OPERA	Open Structure-activity/property Relationship App
OSHA	Occupational Safety and Health Administration
PAH	Polycyclic aromatic hydrocarbon
PBPK	Physiologically based pharmacokinetic
PETA	People for the Ethical Treatment of Animals
PFAS	Per- and polyfluoroalkyl substances
PK	Pharmacokinetic
QSAR	Quantitative structure-activity relationship
SACATM	Scientific Advisory Committee on Alternative Toxicological Methods
SEAZIT	Systematic Evaluation of the Application of Zebrafish in Toxicology (National Toxicology Program)
SOT	Society of Toxicology
Tox21	Collaborative effort among four U.S. Federal Government offices to develop more efficient approaches to predict how chemicals may affect human health
ToxCast	Toxicity Forecaster (U.S. Environmental Protection Agency)
TOXNET	Toxicology Data Network (National Library of Medicine)
TSCA	Toxic Substances Control Act (U.S. Environmental Protection Agency)
U.S.C.	United States Code
USDA	U.S. Department of Agriculture
USGS	U.S. Geological Survey (U.S. Department of the Interior)