Summary Minutes

Scientific Advisory Committee on Alternative Toxicological Methods Meeting

September 28-29, 2021

Virtual Meeting

I. Contents

II. Location of Background Materials and Presentations

Background materials and presentations for the 2021 Scientific Advisory Committee on Alternative Toxicological Methods meeting are available on the National Toxicology Program (NTP) Past SACATM Meetings page (https://ntp.niehs.nih.gov/events/past/index.html?type=SACATM)

III. Frequently Used Abbreviations

3Rs	replacement, reduction, and refinement of animal use
ADME	absorption, distribution, metabolism, and excretion
AOP	adverse outcome pathway
CATMoS	Collaborative Acute Toxicity Modeling Suite
DNT	developmental neurotoxicity
DNTP	Division of the National Toxicology Program (National Institute of Environmental Health Sciences)
DoD	U.S. Department of Defense
EcoWG	ICCVAM Ecotoxicology Workgroup
EPA	U.S. Environmental Protection Agency
FAIR	findability, accessibility, interoperability, and reusability
FAT	fish acute toxicity
FDA	U.S. Food and Drug Administration
GAO	U.S. Government Accountability Office
GLP	Good Laboratory Practice
HSLF	Humane Society Legislative Fund
HSUS	Humane Society of the United States
IATA	integrated approach to testing and assessment
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ICE	Integrated Chemical Environment
ILS	Integrated Laboratory Systems, LLC
IVIVE	in vitro to in vivo extrapolation
MAD	Mutual Acceptance of Data
NAMs	new approach methodologies
NICEATM	NTP Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences
NTP	National Toxicology Program

OECD	Organisation for Economic Co-operation and Development
OPERA	Open (Quantitative) Structure-activity/property Relationship App
OPP	U.S. Environmental Protection Agency Office of Pesticide Programs
ORD	U.S. Environmental Protection Agency Office of Research and Development
PBPK	physiologically based pharmacokinetic
PCRM	Physicians Committee for Responsible Medicine
POD	point of departure
PSCI	PETA Science Consortium International e.V.
QSAR	quantitative structure-activity relationship
SACATM	Scientific Advisory Committee on Alternative Toxicological Methods
SeqAPASS	Sequence Alignment to Predict Across Species Susceptibility
TRUST	transparency, responsibility, user focus, sustainability, and technology
VWG	ICCVAM Validation Workgroup

IV. Attendance

The Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) met virtually on September 28 and 29, 2021. The following individuals participated in the meeting. In addition to participants named below, nearly 160 people viewed the meeting via webcast on September 28, with 120 viewing on September 29.

SACATM Members

Szczepan Baran, VMD, MS, Novartis Institute for BioMedical Research

Joseph Charest, PhD, The Charles Stark Draper Laboratory, Inc.

Amy Clippinger, PhD, PETA Science Consortium International e.V.

K. Nadira De Abrew, PhD, The Procter & Gamble Company (chair)

Sean Gehen, PhD, DABT, Corteva Agriscience

Denis Fourches, PhD, Oerth Bio

Sue Leary, MS, Alternatives Research and Development Foundation

Kathryn Page, PhD, DABT, The Clorox Company

Priyanka Sura, DVM, MS, DABT, ANGUS Chemical Company

Tamara Tal, PhD, Helmholz-Centre for Environmental Research UFZ

Misti Ushio, PhD, TARA Biosystems, Inc.

Ad Hoc SACATM Members

Antonio Baines, PhD, North Carolina Central University

Ellen Berg, PhD, Eurofins Discovery

Adrian Nañez, PhD, Takeda Pharmaceutical Co. Ltd.

Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Principal Representatives

Brian Berridge, DVM, PhD, DACVP, National Institute of Environmental Health Sciences (NIEHS)

Christopher Bever, MD, U.S. Department of Veterans Affairs Office of Research and Development

Brian Cholewa, PhD, National Cancer Institute

John Elliott, PhD, National Institute of Standards and Technology

Suzanne Fitzpatrick, PhD, DABT, U.S. Food and Drug Administration (FDA) Center for Food Safety and Applied Nutrition

John Gordon, PhD, U.S. Consumer Product Safety Commission

Jeanne Goshorn, MS, National Library of Medicine

Steve Hwang, PhD, U.S. Department of Transportation

Anna Lowit, PhD, U.S. Environmental Protection Agency (EPA), ICCVAM Co-chair

Moiz Mumtaz, PhD, Agency for Toxic Substances and Disease Registry

Richard Probst, DVM, MPH, DACLAM, National Institute for Occupational Safety and Health

Barnett Rattner, PhD, U.S. Department of the Interior

Emily Reinke, PhD, U.S. Department of Defense (DoD; acting principal agency representative), ICCVAM Co-chair

Other ICCVAM Representatives

Paul Brown, PhD, FDA Center for Drug Evaluation and Research

Warren Casey, PhD, DABT, NIEHS

William Eckel, PhD, EPA Office of Pesticide Programs

Natalia Garcia-Reyero Vinas, PhD, U.S. Army Engineer Research and Development Center, DoD

Matthew Johnson, DVM, DACLAM, DoD

Nicole Kleinstreuer, PhD, NIEHS

Jessica Leet, PhD, U.S. Department of the Interior

National Institute of Environmental Health Sciences Staff

Robbin Guy

Kamel Mansouri, PhD

Sheena Scruggs, PhD, Designated Federal Official

Mary Wolfe, PhD

Rick Woychik, PhD

NIEHS Support Contractors

David Allen, PhD (Integrated Laboratory Systems, LLC [ILS], contractor supporting the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods [NICEATM])

Patricia Ceger, MS, DABT (ILS, contractor supporting NICEATM)

Parris Milly (Image Associates, contractor supporting the NIEHS Office of Communications and Public Liaison)

Nathan Mitchiner (NETE, contractor supporting the NIEHS Office of Communications and Public Liaison)

Steven Morefield, MD (ILS, contractor supporting NICEATM)

Catherine Sprankle, MS (ILS, contractor supporting NICEATM)

Jonathan Strouse (NTT DATA, contractor supporting the NIEHS Office of Communications and Public Liaison)

Public

Laura Alvarez, MSc, Cruelty Free International

Patience Browne, PhD, Organisation for Economic Co-operation and Development

Tamara Johnson, MS, EPA Office of Pesticide Programs

Vicki Katrinak, Humane Society of the United States/Humane Society Legislative Fund

Carlie LaLone, PhD, EPA Office of Research and Development

Michael Lowit, PhD, EPA Office of Pesticide Programs

Jessica Ponder, PhD, Physicians Committee for Responsible Medicine

Kristie Sullivan, Physicians Committee for Responsible Medicine

Valerie Zuang, PhD, European Commission Joint Research Centre

September 28, 2021

V. Welcome and Opening Remarks

Dr. Nadira De Abrew, The Procter & Gamble Company, chair of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM), called the meeting to order at 10:00 a.m. on September 28. SACATM members and ad hoc participants introduced themselves.

In welcoming remarks, Dr. Rick Woychik, National Institute of Environmental Health Sciences (NIEHS) and National Toxicology Program (NTP) Director, noted the shortcomings of current approaches to toxicity testing, which may be addressed by advancing high-throughput technologies. Developed appropriately, these technologies

have the potential to identify how an individual's genetic background can affect susceptibility to toxicity. They can also provide an approach to modeling both the effects of multiple chemical exposures and the role of the microbiome in affecting toxicity.

In further introductory remarks, ICCVAM Co-chairs Dr. Anna Lowit (U.S. Environmental Protection Agency [EPA]) and Dr. Emily Reinke (U.S. Department of Defense [DoD]) thanked the SACATM members and presenters for their time spend preparing and participating in the meeting. Dr. Nicole Kleinstreuer (NIEHS) noted the role that SACATM plays in advising the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). She further noted that while some brief updates of ICCVAM's activities over the past year would be presented, this meeting would primarily focus on ecotoxicity and new approaches to validation. She acknowledged the contributions and collaborations of international partners, which are important to fostering harmonization of chemical safety testing.

Dr. Sheena Scruggs, (NIEHS), the SACATM Designated Federal Official, read the conflict-of-interest statement and reviewed meeting logistics.

VI. A Year in Review: ICCVAM Accomplishments in Advancing the 3Rs

Dr. Lowit provided an overview of ICCVAM activities over the last year to advance the 3Rs: replacement, reduction, and refinement of animal use in testing. Reviewing the list of active ICCVAM workgroups, she noted ICCVAM's work gets done via these workgroups, which get significant support from the Integrated Laboratory Systems LLC (ILS) contractor staff supporting the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). She reviewed the charges, activities, and publications of each workgroup.

- The **Acute Toxicity Workgroup** are supporting EPA evaluations of in silico models for predicting acute oral toxicity. Work is ongoing to develop similar models for acute inhalation toxicity. A project to evaluate additivity approaches to estimating toxicity of formulations was described in a publication[1] earlier this year.
- The **Ecotoxicology Workgroup** (EcoWG) is compiling a survey of U.S. agency ecotoxicity information needs and uses. This information provides the background needed for identifying relevant new approach methodologies (NAMs), including identification of tests for potential replacement and the policy and regulatory context in which data from those tests are used. The workgroup is also reviewing available NAMs for acute fish toxicity, which was discussed in detail in a later presentation.
- The **In Vitro to In Vivo Extrapolation (IVIVE) Workgroup** is developing a review of IVIVE methods and models used by member agencies. The manuscript will present case studies of how IVIVE has been used in risk assessment. The workgroup is also interacting with the Organisation for Economic Co-operation and Development (OECD) to advance international harmonization of the use of

[1] Hamm et al. 2021. Regul Toxicol Pharmacol. https://doi.org/10.1016/j.yrtph.2021.105007.

IVIVE.

- The **Nanomaterials Workgroup** developed and submitted for publication an agency needs paper[2], which also addresses international efforts to apply NAMs to nanomaterials testing.
- The **Read Across Workgroup** is currently compiling case studies on the application of read-across approaches from the U.S. Food and Drug Administration (FDA), EPA, and the U.S. Consumer Product Safety Commission as a way to avoid animal testing.
- The **Validation Workgroup**, established to update the 1997 ICCVAM "Validation and Regulatory Acceptance of Toxicological Test Methods[3]," will provide details of their activities in a subsequent presentation (see below).

Dr. Lowit summarized 2021 ICCVAM public interactions:

- The annual Communities of Practice webinar focused on non-animal approaches for mixtures assessment[4] (January 2021).
- ICCVAM and NICEATM participated in many activities at the Society of Toxicology meeting[5] (March 2021).
- The ICCVAM Public Forum featured member agencies' presentations about their activities during the year and enabled interactions with the public[6] (May 2021).

Clarifying questions and comments: Responding from a question from Dr. Antonio Baines, North Carolina Central University, Dr. Lowit clarified that the purpose of acute fish toxicity tests is to assess toxicity to fish in their natural environment. Current efforts are focused on reducing or replacing fish use for these endpoints. Acute fish toxicity tests should not be confused with the use of fish models such as zebrafish to predict human toxicity. Dr. Kathryn Page, The Clorox Company, who attended the Public Forum in her role as a SACATM member, read her report of the event[7].

Public Comments

Public comments and SACATM discussion for this topic were combined with those from the following two topics.

VII. Ecotoxicology Testing: Regulatory Needs

Current Ecotoxicology Testing Needs Among Selected U.S. Federal Agencies

Dr. Jessica Leet, U.S. Department of the Interior, presented a summary of the EcoWG's review of ecotoxicology testing needs across federal agencies. She noted that the manuscript in preparation includes 30 authors from seven ICCVAM member agencies

[2] Petersen et al. 2021. ALTEX. https://doi.org/10.14573/altex.2105041.
[3] Available at https://ntp.niehs.nih.gov/iccvam/docs/about_docs/validate.pdf.
[4] Available at https://ntp.niehs.nih.gov/go/commprac-2021.
[5] Available at https://ntp.niehs.nih.gov/go/niceatm-sot21.
[6] Available at https://ntp.niehs.nih.gov/go/iccvamforum-2021.
[7] Available at https://ntp.niehs.nih.gov/ntp/about_ntp/sacatm/2021/sacatm-rpt_iccvam-public-forum-2021_508.pdf.

and ILS, reflecting the broad interest in this issue across the federal government.

Ecotoxicity testing determines hazard or risk presented by substances that may enter the environment. A variety of standardized and internationally harmonized test methods are used for this testing. However, use of animal-based test methods is failing to keep pace with the introduction of new chemicals into the marketplace. There are also concerns about the validity of extrapolating results from the species used in the laboratory to the species of concern. The EcoWG is working to identify federal agency applications and requirements for ecotoxicity testing and to prioritize tests to be targeted for replacement, in line with the goals of the ICCVAM Strategic Roadmap[8].

Emphasizing the breadth and quantity of the information that has been gathered by the EcoWG, Dr. Leet noted that 18 different U.S. statues require or make use of ecotoxicity testing data. These data are generated according to 87 test guidelines that collectively use a relatively narrow selection of surrogate test species. Extrapolation of these results to species in the field creates many uncertainties but advances in computational and in vitro methods provide opportunities to strengthen cross-taxa extrapolation and reduce animal testing. Agencies have also identified opportunities where tests can be waived or bridged. In conclusion, Dr. Leet noted that while there are challenges to the development and use of non-animal ecotoxicology tests, federal agencies remain committed to their development and use in appropriate contexts.

Clarifying questions and comments: Dr. Joseph Charest, Charles Stark Draper Laboratory, asked for clarification of how a species for a test is selected and the basis for applying a cross-taxa extrapolation to data from that test. Dr. Leet referred the question to Dr. William Eckel, EPA Office of Pesticide Programs (OPP), who noted that species for each test are identified in the relevant guidelines, and that he would address this question in more detail in his presentation. Dr. Carlie LaLone, EPA Office of Research and Development (ORD), added that her presentation would describe some bioinformatics approaches for cross-species extrapolation.

Alternate Models for Acute Fish Toxicity Testing: A Survey

Dr. Natalia Garcia-Reyero Vinas, DoD, described the EcoWG's focus on the fish acute toxicity (FAT) test as an example of how to reduce animal use. Aquatic toxicity tests use several species to evaluate the effects of acute or chronic exposure to chemicals and other environmental stressors in different media or environments. The FAT test (OECD Test Guideline 203) is required by many regulatory agencies for registration of new substances. While it has been updated to address animal welfare concerns and encourage use of alternatives for rangefinders, the FAT test still requires a lethality endpoint. That and its widespread use made it of interest to the EcoWG as a candidate for reduction or replacement. The test exposes specific fish species to a stressor for 96 hours and then evaluates mortality or other adverse effects as detailed in the guideline. FAT test data requirements vary by both sector and by geography[9]. Evaluations of in vitro methods as replacements for the FAT test should consider chemical space coverage, performance metrics, and use of proprietary information.

A recent milestone toward replacing the FAT test is the acceptance of the RTGill viability

[8] Available at https://ntp.niehs.nih.gov/go/natl-strategy.
[9] Burden et al. 2020. Environ Toxicol Chem. https://doi.org/10.1002/etc.4824.

assay by OECD as a predictor of acute fish toxicity, a tool for range-finding and prescreening, and an element to be used in a weight-of-evidence approach to hazard assessment. The RTGill viability assay is the first internationally accepted cell-based test for acute fish toxicity. In closing, Dr. Vinas reiterated the alignment of the effort to replace the FAT test with the goals of the ICCVAM Strategic Roadmap and emphasized the need for agencies to evaluate NAM approaches in the context of their own regulatory needs.

Clarifying questions and comments: In response to a question from Dr. Tamara Tal, Helmholz-Centre for Environmental Research UFZ, Dr. Vinas responded that she was unaware of any other in vitro alternatives to the FAT test under consideration, although authorities are considering embryo and cell line-based models. Dr. Sean Gehen, Corteva Agriscience, referencing a presentation graph showing the numbers of vertebrate ecotoxicology studies conducted by contract research organizations from 2014-2017, asked if there has been a similar assessment of the number of vertebrate animals used in these tests. Dr. Vinas was unaware of any assessment but agreed that such data would be of interest. Responding to a question from Dr. De Abrew, Dr. Vinas noted that there has been some consideration of how to incorporate integrated approaches to testing and assessment (IATA) into ecotoxicology, including incorporating information on exposure, linking in vitro assays to key events, and looking at population-level and behavioral effects.

Reduction of Animal Use Through Using Fewer Species

Dr. Eckel reviewed the application of 3Rs principles in recent years by the EPA Office of Pesticide Programs (OPP), focusing on the tests most commonly used. EPA has webpages describing OPP's strategic vision for implementing NAMs, and Dr. Eckel provided specific examples of OPP's 3Rs activities. Many of these activities involve analyses to determine if less data can be used to make equally protective decisions, using computational models to predict toxicity, and modifying test guidelines to refine animal use.

Dr. Eckel described retrospective studies that have supported reduction in animal use. Similar analyses of endpoints that require both acute and chronic tests data may support elimination of the chronic test requirement.

- An examination of data from avian subacute and acute toxicity studies revealed that a robust avian acute risk assessment can be conducted in most cases without the subacute studies[10].
- A study assessing data needs to establish a fish bioconcentration factor led to guidance describing conditions under which a single concentration could be tested, reducing animal use compared to the standard test using multiple concentrations[11].
- An ongoing retrospective study of FAT test data aims to evaluate whether one or two species is consistently more sensitive in this test and thus reduce the number of testing species. The manuscript describing this study is in development.

[10] Hilton et al. 2019. Regul Toxicol Pharmacol. https://doi.org/10.1016/j.yrtph.2019.03.013.
[11] Available at https://www.epa.gov/sites/default/files/2020-07/documents/bcf-study-july-15-2020.pdf.

OPP has applied the Collaborative Acute Toxicity Modeling Suite (CATMoS) quantitative structure-activity relationship (QSAR) tool to predict rat acute oral toxicity and has worked with ORD to improve the Ecological Structure Activity Relationship QSAR tool to predict fish acute toxicity. A paper evaluating CATMoS for predicting pesticide toxicity is planned for publication in 2022 and could provide the basis for future waiver guidance.

Other planned retrospective studies include:

- Evaluating whether the fish acute-to-chronic ratio could be used in place of chronic studies.
- Examining results of fish early life-stage studies to determine whether the full life-stage test is needed.
- Evaluating opportunities to reduce the number of species used in avian reproduction studies.

Clarifying questions and comments: Dr. Page suggested that some of the computational models discussed by Dr. Eckel are sufficiently advanced to replace animal use, and in that regard, recommended that OPP employ them to reduce animal use. Dr. Eckel responded that a major goal of the work he had described was to harmonize with countries that are reducing the number of species used for testing. He acknowledged the value of computational models but noted that there are some chemicals that are not predicted well in models or exhibit anomalous behavior in in vitro tests, and those still need to be tested in animals. Dr. Page asked if data from such animal tests would be used to strengthen the predictive models, and Dr. Eckel responded that that would depend on understanding the reason for the chemical's anomalous behavior. Dr. Tal noted that the Open (Quantitative) Structure-activity/property Relationship App (OPERA) has a model for bioconcentration factor and asked if OPP would consider using that to waive tests. Dr. Eckel replied that OPP uses that as supporting evidence in cases where a judgment needs to be made about toxicity in the absence of experimental data. Dr. Gehen asked whether EPA's emphasis and focus are currently on single chemicals or if they are currently studying mixtures. Dr. Eckel responded that work at OPP and the Office of Pollution Prevention and Toxics is currently focusing on single chemicals, but the Office of Water is studying approaches to assessing mixture toxicity, especially in whole effluents.

Public Comments

Public comments and SACATM discussion for this topic were combined with those from the following topic.

VIII. Ecotoxicology Testing: Research Applications

CATMoS: Acute Oral Toxicity Predictions for Environmental Safety Assessment

Dr. Kamel Mansouri, NIEHS, characterized QSAR models as a resource that can bridge gaps for situations when data are not available as well as reduce animal testing. QSAR models identify features in chemical structures that are correlated with a specific

biological activity. CATMoS[12] is the third and largest of a series of projects that brought global collaborators together to build predictive toxicity models for acute oral systemic toxicity (LD_{50}) and was distinguished by early involvement of regulators to identify relevant endpoints and applications. The CATMoS consensus model leverages the advantages of a variety of model types to expand its applicability domain and improve accuracy. In total, CATMoS combines predictions from about 140 separate models. The predictive performance of the CATMoS consensus model matches the accuracy of replicate in vivo tests for predicting oral acute toxicity outcomes. The consensus model has been implemented in OPERA[13], a suite of QSAR models that predicts properties such as environmental fate, absorption/distribution/metabolism/excretion (ADME) properties, and physicochemical properties. OPERA models are updated regularly as new data become available. OPERA can be used via a web interface or can be downloaded to run locally without connecting to the Internet. OPERA predictions on ~800,000 chemicals are also available via the NTP Integrated Chemical Environment[14] (ICE).

Dr. Mansouri showed an example output from OPERA with consensus predictions for the five acute oral toxicity endpoints (very toxic, nontoxic, EPA hazard categories, GHS hazard categories, and quantitative LD_{50} values). Each prediction has an applicability domain estimate, experimental values where available, and nearest neighbors. NICEATM is collaborating with ICCVAM agency partners to apply CATMoS to various datasets. A collaboration with EPA is examining 178 conventional pesticides classified as EPA Categories II, III, or IV based on animal data. CATMoS predictions have been generated for these chemicals and the hazard classifications are being compared with those based on the animal data, as well as the impact of using the model LD_{50} values on risk assessment conclusions. Next steps will evaluate the applicability of CATMoS estimates as a potential replacement for the rat acute oral single dose study in ecological risk assessments of conventional pesticides.

Clarifying questions and comments: Referring to the process used to develop CATMoS, Dr. Charest asked how data were divided between the training and evaluation sets. Dr. Mansouri explained that a semi-random selection process was used that tried to keep the same distribution of LD50 values and categories between the two sets. Dr. Charest asked if it was possible for a single model to outperform the consensus model. Dr. Mansouri responded that each of the models has strengths and limitations. It's possible that one model could outperform the consensus model in one aspect, but overall the consensus model will perform better and have significantly more coverage across the chemical universe.

In response to a question from Dr. Ellen Berg, Eurofins Discovery, about the potential limitations of CATMoS to predict human toxicity outcomes, Dr. Mansouri acknowledged that the CATMoS model uses rat data to predict rat outcomes but noted that the rat outcome is the regulatory endpoint.

Dr. Tal asked how the quality of the data used to build CATMoS was assessed. Dr. Mansouri answered that both an automated approach and a manual review were used

[12] Mansouri et al. 2021. Environ Health Perspect. https://doi.org/10.1289/EHP8495.
[13] Available at https://ntp.niehs.nih.gov/go/opera.
[14] Available at https://ice.ntp.niehs.nih.gov/.

to identify erroneous data. He added that after the consensus model was built, some of the consensus predictions were compared to the training data and used to identify further errors, such as incorrect decimal point placement or unit errors. Dr. Tal then noted that while the balanced accuracy and specificity of the consensus models for making categorical predictions were very good, the lower performance for sensitivity suggests there is some opportunity for improvement in the model's ability to identify true positives. Dr. Mansouri responded that the evaluation was separate for each category and depended on the availability of chemicals in that category for comparison. The performance assessment of the consensus model is discussed in greater detail in the paper[15], which might provide more insight into this question.

The Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) Tool: Catalyzing a Change in Species Extrapolation

Dr. LaLone, EPA ORD, described tools to derive ecological points of departure (PODs). For ecotoxicity testing, surrogate species are used and often chosen for reasons such as easy husbandry, availability, short life spans, and so on. Data from these species are then used to predict effects on other species, a process known as cross-species extrapolation that EPA is seeking to improve. Extrapolation is important because testing resources are limited and it's impractical to test all species of interest on all chemicals of interest. A key aspect of extrapolation is chemical sensitivity, which is affected by properties such as life stage, ADME, and toxicodynamics. EPA is using bioinformatics to explore how these properties are conserved across species.

One key aspect of chemical sensitivity is target receptor availability. Protein sequence information can be easily compared across species to identify the availability of a specific receptor and predict susceptibility on that basis. EPA has developed a tool, Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS), that evaluates the degree of conservation in a primary amino acid sequence and identifies amino acids that affect functions such as hydrogen bonding and ligand binding. The latest version of SeqAPASS links susceptibility predictions with a knowledge base of ecotoxicity data (ECOTOX Knowledgebase). The key input for SeqAPASS are the molecular target for the chemical of interest and a known sensitive or target species. SeqAPASS does not predict the degree of sensitivity or susceptibility but gives a yes/no answer as conserved or not conserved. Advantages of SeqAPASS include its ability to leverage new tools and technologies and improvements in availability of sequence data. It is publicly available, encompasses hundreds or thousands of species, guides users to appropriate input, and has evolved to incorporate user-friendly advances in bioinformatics.

Dr. LaLone described an example of how SeqAPASS has been used to extrapolate the applicability of an adverse outcome pathway from Apis bees to non-Apis bees. It has also been used to extrapolate high-throughput screening data and predict bioaccumulation. Future applications of SeqAPASS will explore how its predictions can connect to other data resources such as the EPA ECOTOX Knowledgebase or to systematic literature reviews. SeqAPASS developers are also advancing structural evaluations by generating docking and virtual screening models and partnering with

[15] Mansouri et al. 2021. Environ Health Perspect. https://doi.org/10.1289/EHP8495.

laboratories to generate confirmatory experimental data. To support development and application of resources such as SeqAPASS, EPA participates in the International Consortium to Advance Cross Species Extrapolation in Regulation.

Dr. LaLone closed her talk by providing an overview of related research efforts at ORD. She described approaches to establish PODs that use transcription data that are being compared to apical POD values to understand the value of transcriptional POD. These approaches largely use data from human-based assays, but EPA is adapting this approach for ecotoxicity. The ORD laboratory in Duluth is developing high-throughput assays based on four species representing the three major trophic levels in ecosystems: primary producers (plants), primary consumers (herbivores), and secondary consumers (omnivores/carnivores). The BMDExpress tool (Sciome) uses data from these assays to derive a transcriptomics-based POD. Comparing data from the different species allows identification of PODs that are less than or equal to the most sensitive chronic endpoint. This approach is being explored using cell lines for ecologically relevant species, and transcriptomics-based PODs for ecotoxicology have been presented as a case study to the international consortium Accelerating the Pace of Chemical Risk Assessment.

Clarifying questions and comments: Dr. Page asked if SeqAPASS can interact with EPA's Web-based Interspecies Correlation Estimation[16] tool. Dr. LaLone responded yes, that her group has collaborated with other groups within ORD to compare SeqAPASS predictions with empirical data to better support evidence for conservation of targets across species. Dr. Page asked how involved state agencies are in this work, and Dr. LaLone noted that her group liaises with the Minnesota Department of Health and a roundtable group of state agencies that interacts with EPA. They also present at meetings such as the Society for Environmental Toxicology and Chemistry and the Society of Toxicology. Responding to Dr. Page's question about how mixtures are being considered, Dr. LaLone commented that they are currently looking at one target at a time, but that they can look at multiple targets that might be relevant in a mixture and combine that data. Referring to Dr. LaLone's statement that SeqAPASS provides a yes/no answer on sensitivity and susceptibility, Dr. Gehen asked if that was derived in a binary or probabilistic manner. Dr. LaLone responded that it is based on the percent similarity comparing sequences, and they set a default cutoff based on the lowest percent similarity where ortholog candidates are identified in the data set. Incorporating more advanced methods such as docking models presents a potential opportunity for improving predictions. The key piece of information for these analyses is knowing the molecular target for the chemical of interest.

Public Comments

One written public comment was submitted for this section, on behalf of the Humane Society of the United States and the Humane Society Legislative Fund (HSUS/HSLF).

Oral Public Comments

Ms. Vicki Katrinak, representing HSUS/HSLF, was pleased to note this meeting's focus on ecotoxicity. Traditional tests for these endpoints are subject to the same drawbacks as animal tests for human safety. The FAT test is highly variable and poorly predicts toxicity of target species. She welcomed the OECD's acceptance of the RTGill assay

[16] Available at https://www3.epa.gov/webice/.

and encouraged agencies to consider acceptance of tests based on fish cell lines as a replacement for animal tests. The large number of fish used in traditional tests should make this assay a priority for replacement and reducing the number of species required is a promising approach. HSUS/HSLF encourages EPA to continue conducting retrospective analyses to identify waiver opportunities and would like to see other agencies engaging in such activities.

Dr. Jessica Ponder, representing the Physicians Committee for Responsible Medicine (PCRM) commended ICCVAM for their activities and communications, including 18 publications so far this year. Accomplishments of note include the CATMoS publication and acceptance of the guideline for defined approaches to skin sensitization. She praised continued activities around characterizing variability of reference animal data and the cooperation that is making these studies possible. Agencies should update guidance or publish policy documents to encourage or highlight opportunities for use of new methods. Dr. Ponder was pleased to see support for the idea that three species are not needed for the FAT test and the broader use of bioinformatics approaches for ecotoxicity risk assessment, and she encouraged agencies to consider how in vitro and in silico data can be used in decision making to reduce animal use requirements. Federal agencies should also encourage acceptance of new approaches by state agencies. The challenge currently faced by toxicology is to characterize risks of new chemicals in real time so that risks can be addressed proactively rather than reactively. This will be possible via high-throughput screening and in silico and artificial intelligence approaches. Read-across methods should be used to generate more comprehensive risk assessments, and NAM data should be used alone rather than as a supplement to animal data.

Comments from Designated SACATM Discussants: ICCVAM Year in Review

Discussants for “ICCVAM Year in Review” were asked to consider the following questions:

- Are there endpoints or specific test methods that could be considered for prioritization by ICCVAM?
- What topics do you suggest be the focus of future ICCVAM Communities of Practice webinars?
- What are your suggestions on other ways to engage ICCVAM stakeholders and foster partnerships between governmental and nongovernmental groups?
- What are the areas/topics that you feel are not being adequately addressed by ICCVAM workgroups?

Dr. Page, first discussant, felt that a lot of progress has been made in advancing alternatives for acute toxicity endpoints. She would like ICCVAM to continue to work on endpoints that use the most animals. A major issue faced by the regulated community is the lack of knowledge about the available alternatives and how they can be applied in different settings. To that end, a future Community of Practice webinar could focus on computational approaches with a broad view of what resources exist and how they could be applied to fulfill information needs of regulatory agencies that currently require animal testing. Regarding fostering partnerships, Dr. Page noted EPA’s effectiveness at

organizing stakeholder groups. More agencies should engage in meetings with stakeholders to discuss implementation of NAMs. Such meetings should be open to all interested parties and facilitate collaborative work to gain acceptance of NAMs that will actually be used. Regulators should also reach out to stakeholders that are not using available NAMs and find out what issues need to be addressed. Identifying and publicizing case studies could help with this. She felt that ICCVAM is doing a good job of setting tasks and expectations for their workgroups and noted the usefulness of the scoping documents they have published. She felt that the effectiveness of the workgroups could be improved by incorporating consideration of mixtures into their initial charges. Dr. Lowit responded that ORD is actively working on building training modules that are going to engage different audiences at different levels.

Dr. Misti Ushio, TARA Biosystems, Inc., second discussant, noted that this session effectively showed the breadth of the work in this area, and the number of publications issued is impressive. Focusing on fostering partnerships, she felt that a priority activity should be to encourage industry and commercial entities to accept and use existing technologies. This could be done through coordination of efforts by existing organizations with regulators to identify barriers and harmonize efforts to reduce duplication of efforts. Referring to the report of the 2018 acute toxicity workshop[17], she wondered if there were lessons learned from that workshop and whether it can be used as a roadmap. Dr. Kleinstreuer responded that that publication demonstrates how to engage stakeholders early so that new approaches can be developed to meet their needs. That was the purpose of the workshop and a model that will be followed in future activities.

Dr. Baines, third discussant, noted that animal testing is a concern raised by students in his toxicology classes. Modeling of mixtures should be a priority for future work because that represents a more realistic exposure scenario. Alternatives need to incorporate both genetic variability such as that found among different rat strains and variability due to other factors such as the microbiome. He encouraged the continuation and broadening of collaborations with an eye toward involving students and minority-serving institutions. He also stressed the need to explore how toxicogenomics can be leveraged to represent diversity.

Dr. Adrian Nañez, Takeda Pharmaceutical Co. Ltd., fourth discussant, noted that drug companies utilize 3Rs approaches internally, and he wondered how to encourage these companies to share best practices to reduce animal use. Anything that could be done to improve accessibility of data would be useful.

Additional SACATM Comments

Dr. De Abrew wondered whether agencies survey both the method developers and the users of alternative methods, and what they asked. Dr. Lowit answered that EPA talks to both regulators and researchers. She noted that some agencies lack a regulatory component and others lack a research component. The ICCVAM workgroups bring together volunteers from agencies to look at information needs and alternative use across a variety of contexts, and the survey papers they develop try to capture that complexity. Methods developers use these papers to conceptualize their work and start

[17] Kleinstreuer et al. 2018. Comput Toxicol 8:21-24. https://doi.org/10.1016/j.comtox.2018.08.002.

discussions with regulators.

Responding to Dr. Ponder's comments, Dr. Page agreed that she would like to see NAMs applied to chemical exposures experienced by disadvantaged populations and caused by natural disasters due to climate changes. She also encouraged NAMs developers to incorporate human diversity into testing models. The availability of NAMs needs to be broadly publicized so they can be applied beyond their original scope as appropriate. Dr. Tal reiterated the importance of education and outreach, and suggested targeting communications to late-adopting agencies.

Comments from Designated SACATM Discussants: Ecotoxicology Testing: Regulatory Needs

Discussants for "Ecotoxicology Testing: Regulatory Needs" were asked to consider the following questions:

- After acute fish toxicity testing, which ecotoxicity test methods could be considered as the next priority(ies) for replacement?
- What other analyses or approaches are available to support the use of fewer animal species for acute fish and bird toxicity testing?

Dr. Gehen, first discussant, appreciated this meeting's focus on ecotoxicology; there are a lot of species that need to be protected in this sphere. It is important not to compromise protection while minimizing animal use, and ICCVAM is doing a good job of striking that balance. Noting the renewed global interest in testing for endocrine disruptors, Dr. Gehen felt that these efforts have the potential to use a lot of animals: alternatives need to continue to be developed and used rather than just accepting a proliferation of animal testing for this purpose. While it is worthwhile to work toward a replacement for the FAT test, it is important to ensure that ongoing animal studies are being fully utilized by adding endpoints such as 'omics that can reduce the need for follow-up studies with other species. An important element of this will be to develop a more mechanistic understanding of toxicity in environmental species, which will help reduce reliance on whole animal testing.

Ms. Sue Leary, Alternatives Research and Development Foundation, second discussant, reiterated the need to replace the FAT test because it uses so many animals. Whole effluent toxicity testing is estimated to require six million animals per year. The adoption of the RTGill test by OECD is an important advance and might be applied to integrated testing strategies to meet regulatory information needs. Other replacement projects could focus on chronic and reproductive tests such as the fish early life-stage and the bioaccumulation test. Some strategies can include combining tests and using adverse outcome pathways (AOPs) to build testing strategies. Ms. Leary welcomed efforts to reduce the number of species required in tests. EPA has demonstrated the effective use of testing waivers, and more agencies should implement these. She closed by noting a trend for increasing data requests and expressing the hope that the default for addressing these will not be more animal testing.

Additional SACATM Comments

Dr. Page appreciated the work being done to reduce animal use but encouraged agencies to move toward implementation of non-animal alternatives.

Dr. Tal encouraged the development and adoption of assays currently under consideration by OECD that use zebrafish embryos to evaluate developmental neurotoxicity (DNT).

Dr. Charest noted that material in the background readings seemed to indicate that there has been some success in modeling bioaccumulation in vitro and in silico, and this endpoint may be worth exploring in the near term. Dr. Eckel agreed but noted that while modeling bioaccumulation is straightforward for certain types of chemicals, it would be more difficult for chemicals that have properties outside the domain of the predictive models.

Responding to points made about DNT, Dr. Lowit noted that regulatory agencies are moving acceptance of alternatives for this endpoint forward. EPA is developing case studies using in vitro NAMs in collaboration with the NIEHS Division of the National Toxicology Program (DNTP). There's a strong consensus that animal studies don't correlate with human epidemiology and that human cell-based assays may address the relevant questions more effectively.

Comments from Designated SACATM Discussants: Ecotoxicology Testing: Research Applications

Discussants for "Ecotoxicology Testing: Research Applications" were asked to consider the following questions:

- What is needed to increase the confidence across sectors in using CATMoS on a routine basis to identify potential acutely toxic substances?
- Please comment on the practical application and functionality of CATMoS and SeqAPASS. What recommendations do you have for improving their application and functionality?

Dr. Clippinger, PETA Science Consortium International, first discussant, complemented the CATMoS team on the planning and execution of the project. She especially appreciated consideration of the variability of the animal test in the discussion of the model's performance. Additional case studies using CATMoS should be funded and developed, specifically including chemicals nominated by ICCVAM agencies. Companies should also apply these models to their own chemicals and share their results. In particular, data need to be generated to build confidence that any model put into regulatory use will identify very toxic chemicals. Dr. Clippinger expressed a hope that agencies would accept data from these models for high-confidence classifications while data continue to be generated to support their use for other classifications. It would also be useful to get recommendations on how to use CATMoS in an IATA to understand the mode of action for specific chemical classes. Training of regulators is very important, and it sounds like some of this is under way. Stakeholder discussions should emphasize the benefits of NAMs to increase comfort with changing practices. Clear policies from regulatory agencies would also help with adoption. Finally, she emphasized the need to continue cross-agency and international discussions.

Dr. Tal, second discussant, felt that CATMoS was a good example of agency involvement in NAM development and collaboration to build a strong consensus model. The EPA-NICEATM collaboration to test chemicals of interest represents a good approach to build confidence with end users. She expressed an interest in seeing some

clarification on the limitations of the CATMoS model for ecotoxicity predictions, and wondered if incorporation of metabolism, additional physicochemical properties, or high-throughput screening data would capture more mechanistic information. Turning to the question of application, Dr. Tal reiterated the need for hands-on training. She welcomed the availability of the CATMoS predictions via ICE and complemented the ICE team on the recent improvements in usability and the launch of the Chemical Quest tool. However, ICE needs a training guide and open access to relevant articles to help users understand more about how models are generated and what the data mean. There is also a need to publicize examples of how CATMoS has been used in regulatory applications, and she encouraged the development of reference lists for both CATMoS and OPERA. Reference chemical lists for DNT and immunotoxicity, including negatives, would also be helpful. Regarding the application and functionality of SeqAPASS, the case studies developed so far have effectively supported its use for cross-species extrapolation. The next step should be experimental evaluation of these predictions in ecologically relevant models to demonstrate that, for example, certain taxa are not more sensitive than predicted. Experimental evaluations could also support development of approaches to incorporate existing human and rat high-throughput data in a weight-of-evidence assessment. Development of additional case studies for regulatory applications would also be useful, and information about all these activities and resulting publications should be shared on the SeqAPASS website. The incorporation of docking models and 3D models into SeqAPASS holds a lot of promise. She suggested linking SeqAPASS predictions in AOPs via the OECD wiki, as there is potential for application of this technology for identifying key events. Other experiments that could be carried out include gene editing studies to identify elements conserved across species. She closed by expressing her enthusiasm for the transcriptional POD studies.

Additional SACATM Comments

Dr. Mansouri noted that NICEATM has offered training on CATMoS via the recent "Nix the Six" webinar series[18].

In response to Dr. Tal's comments, Dr. LaLone noted that site-directed mutagenesis is being used to explore the effects of mutations in specific amino acids. Cell-based assays are being used for high-throughput transcriptomics approaches. She agreed on the importance of documenting use of SeqAPASS. Google Analytics is tracking who is using the tool, and this information will be used to further promote the tool. The next planning cycle for SeqAPASS development is currently underway, and topics being discussed include defining taxonomic domains of applicability and looking at how SeqAPASS can be used to walk down an AOP for an endpoint such as honeybee colony failure. Experimental data are also being used to strengthen some of SeqAPASS' default settings.

Dr. De Abrew asked how genetic variation is characterized in wildlife species. Dr. LaLone responded that this is a topic that needs to be better understood. Dr. De Abrew then asked about the relationship between conservation of a primary protein sequence and conservation of the 3D structure. Dr. Lalone responded that studies are ongoing

[18] Webinar series information available at https://www.pcrm.org/ethical-science/animal-testing-and-alternatives/nura; CATMoS video available at https://pcrm.widen.net/view/video/qx5zu7mgk2/Session-1-Recording-Nix-the-Six?x.share=true&x.portal_shortcode_generated=5fgd9gox&x.app=portals.

using x-ray crystallography and site-directed mutagenesis to try to characterize that relationship quantitatively with respect to chemical interaction. That said, sequence-based approaches have been found to be fruitful and this simpler approach could support broader understanding and acceptance, with structural comparisons representing an added benefit.

Dr. Page asked Dr. Mansouri about the extent to which CATMoS could replace animal use for acute oral toxicity data requirements for human health, and how a similar approach might be applied to acute inhalation toxicity. Dr. Mansouri replied that CATMoS can be applied to any setting where an acute oral toxicity LD_{50} is required. While NICEATM's current work focuses on pesticides, application could be broader. The same modeling approach is going to be used for inhalation toxicity, though the exact project parameters are still being determined and data collection is still ongoing. Dr. Lowit added that the most difficult task in pesticide regulation is predicting the LD_{50} number. Predicting how a chemical would fall into specific EPA or United Nations Globally Harmonized System classification categories is less challenging but presents the problem of how to deal with discordant categorization.

Dr. Page then noted the need to consider how these methods could be used to assess toxicity of mixtures, both formulations and environmental mixtures. One part of this is to identify the constraints of a method up front and how they could affect a model's applicability for certain types of mixtures.

Dr. Gehen noted that the consensus model was based on rodent LD_{50} data and wondered about its applicability to other species. Specifically, he wondered whether new models would need to be built for ecological species. Dr. Mansouri acknowledged that CATMoS predictions may not be directly applicable to other species, but there is the potential for extrapolation. Dr. Eckel noted that CATMoS doesn't account for differences in toxicity due to sex or stereochemistry, which are other limitations to be aware of. Dr. Gehen noted that bioavailability would be another factor to consider in species extrapolation, for example the difference of oral absorption vs. absorption through gills.

Returning to the question of mixtures, Dr. Kleinstreuer noted a recent publication[19] that describes application of CATMoS in combination with the additivity formula for prediction of mixtures toxicity developed by the United Nations Globally Harmonized System for Classification and Labelling of Chemicals. DoD used this approach to predict hazard classification for 500 mixtures and concluded that it was valid.

Dr. De Abrew thanked the day's presenters and discussants and felt that the day's focus on ecotoxicity led to some fruitful discussion on this topic. He adjourned the meeting for the day at 3:32 p.m.

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Dr. De Abrew called the second day of the meeting to order at 10:00 a.m. SACATM members, ad hoc members, key DNTP staff, and the ICCVAM co-chairs introduced themselves. Dr. Scruggs reviewed meeting logistics and read the conflict-of-interest statement.

[19] Chushak et al. 2021. Chem Res Toxicol. https://pubmed.ncbi.nlm.nih.gov/33206501/.

IX. Evolving Approaches to Validation

ICCVAM Validation Workgroup Update

Dr. John Gordon, U.S. Consumer Product Safety Commission, provided an overview of the activities of the ICCVAM Validation Workgroup (VWG), which has representation from nine ICCVAM agencies. The workgroup is updating the ICCVAM document "Validation and Regulatory Acceptance of Toxicological Test Methods," which was published in 1997. While much of the information in the document remains relevant, some of it is outdated. Topics to consider for the update include:

- Fostering the use of efficient, flexible, and robust practices to establish confidence in new methods.
- Developing and evaluating flexible practices that consider context of use to build confidence in new methods, including biological and mechanistic relevance to appropriate taxa, consideration of relevant reference data, and case studies/examples.
- Advancing recommendations to facilitate regulatory acceptance through understanding regulatory needs and decision contexts, application of fit-for-purpose approaches within regulatory context, intra- and interagency coordination and harmonization, and communication and training. Inclusion of regulatory affairs people in this area will be very important.
- Determining how new principles of validation support global harmonization.
- Referencing relevant documents published since 1997.
- Outlining best practices to support sensitivity, reproducibility, and robustness.
- Examining best practices for quality and quality systems development such as incorporation of control charting tools, in-process control measurements, and flowcharts; developing statistical models; and setting specifications.

Summarizing the status of the revision effort, Dr. Gordon stated that the format and organization of the document is still under development. The VWG and NICEATM staff will contribute to drafting and editing, after which ICCVAM members will review and provide comment. There will also be opportunities for stakeholders to comment on the document. This is intended to be a living document and the VWG will develop suggestions for periodic update.

Clarifying questions and comments: Responding to a question from Dr. De Abrew, Dr. Gordon indicated that the intended audience for this document would include both regulators and stakeholders. ICCVAM envisions that this document will both help regulators develop guidance and help test method developers understand how to interact with regulatory agencies. The goal is to encourage people to think beyond traditional approaches to validation. Responding to questions from Dr. Tal and Dr. De Abrew, Dr. Gordon noted that the revised document would address both harmonization of information requirements across federal agencies and international harmonization.

An OECD Perspective on Building Confidence for New Approach Methodologies

Dr. Patience Browne, OECD, stated that the OECD considers Good Laboratory Practice (GLP) and harmonized test guidelines to be the primary pillars supporting Mutual Acceptance of Data (MAD), a legally binding international agreement that member countries accept data resulting from an OECD Test Guideline conducted in a GLP lab. The standards for MAD specify rigorous processes for GLP quality assurance and for scientific validation, which Dr. Browne described in detail. Importantly, MAD prevents repeat testing but does not prevent an interpretation that could include additional data to come to conclusions on chemical hazards. OECD's Guidance Document 34[20] provides guidance for method validation; Guidance Document 34 focuses primarily on in vivo methods but is applicable to in vitro methods as well. The advantages to the current MAD paradigm include buy-in from a broad range of experts and demonstrated reduction of duplicative testing, currently estimated to be approximately €300M annually. Disadvantages of the current paradigm include the time-consuming processes and fixed OECD review schedules. Dr. Browne highlighted the recent adoption by OECD of Guideline 497 for defined approaches for skin sensitization testing[21]. This represents a new kind of Test Guideline for OECD and should serve as a blueprint for other defined approach guidelines.

OECD is engaged in several activities supporting establishing confidence in new methods that reflect the rigor of the current MAD principles. A working group on Good Computational Methods was established to consider how MAD could be expanded beyond traditional experimental laboratory data. Initial discussions within the working group agreed that it is possible to carry out computational approaches in a GLP environment but that may not always be necessary to achieve regulatory acceptance. Other important factors affecting the regulatory acceptance of computational methods include reproducibility and transparency of the workflow. Standardized reporting templates and formats that are necessary under MAD already exist for IATAs and QSAR models and will soon be available for emerging technologies such as 'omics. Dr. Browne reviewed the categories for OECD Harmonised Templates[22] for reporting chemical test results, of which 130 are currently available. The various standardized templates help to assure consistency and quality of data and allow for establishment of a structured database for storage, sharing, and access of in vitro and in vivo chemical data. The data structure is critical for assuring interoperability among OECD and non-OECD electronic tools.

Turning to the question of scientific validity, Dr. Browne described the OECD IATA Case Studies Project[23]. The goal of this project is to exchange information, create a common

[20] "Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment", available at https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2005)14&doclanguage=en.

[21] Available at https://www.oecd-ilibrary.org/environment/guideline-no-497-defined-approaches-on-skin-sensitisation_b92879a4-en?_ga=2.92781918.297652564.1633974148-2026927626.1632762805.

[22] https://www.oecd.org/ehs/templates/

[23] Available at https://www.oecd.org/chemicalsafety/risk-assessment/iata-integrated-approaches-to-testing-and-assessment.htm.

understanding, and provide a possible pathway for NAMs to be accepted in test guidelines, defined approach guidelines, and testing strategies or batteries. So far, 24 case studies have been published with eight case studies under review this year. The case studies and lessons learned from each review cycle are published annually[24].

Considering how stakeholders can start using NAMs for regulatory purpose in a stepwise fashion, Dr. Browne noted the many resources are already available. These include OECD's guidance documents on QSAR[25] and good in vitro methods practices[26]. Both of these documents establish standards for documentation and review that countries could opt into as appropriate for their specific legal requirements. For IATAs, the testing is well-vetted in many cases, but their application and assessment needs to be clarified. Ultimately, these may not be covered by MAD but could still be regulatorily accepted in specific scenarios. Dr. Browne reviewed steps that could be taken to build confidence in NAMs, including description of the applicability domain, characterization of uncertainty, and agreed-upon vocabularies for methods, effects, and endpoints. Considerations of how to demonstrate performance should include choice of reference data and physiological validation, especially for complex models like organs-on-chips.

In closing, Dr. Browne suggested that some practical perspectives are needed on how to take up innovative approaches in a regulatory context. Harmonized test guidelines are valuable, but they are certainly not the only solution. Consideration of how to use data generated in alternative methods research that may not become test guidelines is needed. Rather than asking if methods are ready for regulatory use, it might be more useful to ask what is missing from the "confidence checklist."

Clarifying questions and comments: Dr. Page asked Dr. Browne what OECD lessons learned would benefit ICCVAM in the promotion of harmonization. Dr. Browne responded that harmonization is a challenge, but one promising approach is to identify areas of common ground. In response to a question asked by Dr. Gehen about ways to streamline the OECD review process, specifically with respect to NAMs, Dr. Browne replied that, within the test guidelines program, availability of sufficient data and identification of a clear regulatory application that addresses global requirements can help speed the review process. Outside of the test guidelines program there's more flexibility in the review process, but a clearly articulated regulatory application is still helpful. In addition, for IATAs which are designed to address a specific regulatory scenario there isn't the same need for consensus on the approach among all member countries as with the Test Guidelines which are governed by MAD, and there's a greater ability to accommodate proposals that may not be globally relevant. Dr. Denis Fourches, Oerth Bio, asked Dr. Browne to expand on her comments about the need for consistency of nomenclature, especially in the context of next-generation reagents such as RNA interference reagents. Dr. Browne noted that OECD has a document about

[24] Report of the 2019 review cycle (published Dec. 2020) available at https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2020)24&doclanguage=en.
[25] Available at https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?doclanguage=en&cote=env/jm/mono(2007)2.
[26] Available at https://www.oecd.org/env/guidance-document-on-good-in-vitro-method-practices-givimp-9789264304796-en.htm.

RNA interference reagents under development right now. More broadly, the concept of harmonized terminology is of interest because of its potential to support interoperability of databases. She acknowledged that OECD has not been very meticulous about this in the past; for example, the same endpoint is referred to using different terminology in different test guidelines. Current efforts are focusing on harmonizing terminology within OECD resources such as test guidelines, OECD QSAR Toolbox, the AOP Ontology Project, and projects around IATAs. Subsequent steps will coordinate these efforts with similar efforts in the U.S. and Europe. Emerging technologies are a focus because there's an advantage to harmonizing terminology up front rather than doing it retroactively. Dr. De Abrew asked if there was an intention within OECD to develop case studies describing applications of connections between OECD data resources and other resources. Dr. Browne responded that right now, chemical safety data are housed in separate resources such as the EPA Chemicals Dashboard in the U.S. and the International Uniform Chemical Information Database in Europe. A global chemicals safety database could be a central repository for such data and eventually become an AOP/NAM generator that would support the development of predictive models. Expanding on this, Dr. Kleinstreuer noted that developers of the NTP ICE resource are digitizing and extracting data from legacy DNTP studies and annotating them using controlled vocabularies, including some from OECD harmonized template reporting formats. She also noted that OPERA is developing a plug-in to OECD Toolbox.

Implementing the Metrics Workgroup Recommendation: Use of Validated Alternatives

Dr. Paul Brown, FDA Center for Drug Evaluation and Research, summarized the work of the ICCVAM Metrics Workgroup, which culminated in the publication of the document "Measuring U.S. Federal Agency Progress Toward Implementation of Alternative Methods in Toxicity Testing[27]" earlier this year. Creation of the Metrics Workgroup was prompted by a 2019 report on animal use in research by the U.S. Government Accountability Office (GAO). The GAO recommended that ICCVAM establish a workgroup to develop metrics for assessing progress on the development and promotion of alternatives to animal use. They also recommended that ICCVAM incorporate those metrics on adoption of alternatives into public reports.

The Metrics Workgroup found that regulated industries can and do take steps to reduce, replace, or refine animal use, though much of these efforts take place in the pre-regulatory arena. U.S. agencies can encourage use of alternatives and do so in a variety of ways. However, they do not have the authority to ban the use of animal methods. Many statutes require submission of all available data, and animal data are still submitted whether they are required or not. Dr. Brown noted that there are in vitro and in silico methods in use and accepted by agencies, but few alternatives exist that are capable of completely replacing an animal test directly on a one-to-one basis, especially for tests that include repeated doses, implantation, or multiple endpoints.

The Metrics Workgroup's key conclusion was that there is no one set of metrics that can be used by all ICCVAM member agencies to measure implementation of alternatives or reduction of animal use. The workgroup recommended that agencies should develop

[27] Available at https://ntp.niehs.nih.gov/iccvam/docs/about_docs/iccvam-measuringprogress-feb2021-fd-508.pdf.

their own metrics relevant to their unique situations. These could include qualitative and quantitative metrics used to assess progress, and these should be communicated to the public.

Dr. Brown concluded by presenting examples of information that is relevant to FDA's efforts to advance alternatives. The FDA Alternative Methods Working Group has established a website[28] that provides access to publications and presentations and lists definitions related to alternatives. The Working Group reports annually on FDA activities in the area, including work done with other federal agencies and international agencies. The most recent edition[29] of this report was published in January. The Working Group also coordinates an internal FDA webinar series on alternative methods, to allow test method developers to explain to reviewers how an alternative might be applied and how to interpret its data.

Clarifying questions and comments: Dr. Clippinger noted that agencies such as EPA have published specific information on animals saved, NAMs accepted, and the types of studies most often requested by regulators. She asked if there are plans to continue the Metrics Workgroup to encourage these types of activities among other agencies. Dr. Brown responded that the Metrics Workgroup hasn't met since publication of their report but that is an activity that could be considered. He stressed the importance of establishing metrics that are meaningful and noted that some agencies don't get submissions so there's nothing to count, or may get numbers but don't have the context to determine exactly what they mean. Ms. Leary asked if it would be possible to have reports from each agency on their plans in this area in time for next year's Public Forum. Dr. Brown replied that the workgroup recommended that, and what happens next is up to the agencies. He noted the progress represented by creation of public webpages by agencies.

New ICCVAM Activities for 2021-2022

Dr. Casey introduced his topic by noting that hazard classification categories were developed using animal data, which puts human-based data at a disadvantage. This approach does not consider animal variability, which causes difficulty with validation of NAMs intended to replace the animal test. A groundbreaking paper on eye irritation testing by Clippinger et al.[30] proposes another approach by suggesting a mechanistic, human biology-based approach for assessing method relevance. The approach draws parallels to the animal method but does not require concordance. Key points made in the paper include:

- When there is discordance between human-based NAMs and the rabbit test, findings from in vitro and ex vivo systems should carry more weight than rabbit data.
- The scientific validity of an in vitro or ex vivo method should be assessed by understanding the assay's relevance to human biology and mechanisms of eye

[28] Available at https://www.fda.gov/science-research/about-science-research-fda/advancing-alternative-methods-fda.
[29] Available at https://www.fda.gov/media/144891/download.
[30] Clippinger et al. 2021. Cutan Ocular Toxicol. https://doi.org/10.1080/15569527.2021.1910291.

irritation.

- Ultimately, a replacement method that provides a model grounded in human biology will be as good or better at protecting human health than the currently used rabbit test.

Dr. Casey reviewed the list of current ICCVAM workgroups and expert groups. Workgroups are defined as having an active charge and current activities, while expert groups have no ongoing activities but are comprised of ICCVAM agency scientists with a common interest in order to keep in touch and share information. Activities of workgroups are described on the NTP website. A new workgroup, the Consideration of Alternative Methods Workgroup, will address the issue of incentivizing and providing support for replacement of animal models with alternatives. The workgroup will organize public workshops to get public input on approaches to address this challenging problem.

Dr. Casey then turned to the question of how to help test method developers understand regulatory testing requirements. Rather than developing non-animal tests to replace one specific test, it would be better if testing requirements across agencies were understood and communicated more broadly so sets of information requirements could be addressed by groups of alternative methods. He suggested that the era of identifying alternatives for acute toxicity tests is coming to an end, and remaining endpoints are going to be more challenging to develop alternatives for. He closed by referring to the 2018 Strategic Roadmap and noting that it continues to drive ICCVAM activities.

Clarifying questions and comments: Referring to Dr. Casey's comment about researchers used to working with animal models finding it difficult to adopt non-animal alternatives, Dr. Gordon and Dr. Priyanka Sura, ANGUS Chemical Company, both noted that this issue is faced by both regulators and regulated industry. Dr. Casey agreed and acknowledged that past communication efforts have failed to address this problem. Interests of all those groups will be considered as plans for the workshops are made.

Public Comments

Three written public comments were submitted for this section, on behalf of HSUS/HSLF, PCRM, and the Institute for In Vitro Sciences.

Oral Public Comments

Ms. Kristie Sullivan, PCRM, expressed appreciation for the flexible process toward validation that is being embraced by the VWG. A more flexible validation approach could reduce instances of difficulty with implementation of methods that have been nominally accepted. She cautioned against the possibility of evaluating the same methods repeatedly for slightly different purposes and suggested this problem could be avoided by harmonizing decision contexts and processes across agencies. Characterizing data sets used to validate a method by chemical properties rather than use cases could also support broader acceptance of the method. Finally, when an evaluation is complete, agencies should publish clear policies that describe the contexts under which a new approach can be used. She expressed appreciation for the VWG's stated intention to solicit stakeholder feedback on the revised document and hoped that the document would incorporate agency-specific information about nomination and evaluation of

NAMs. She agreed with Dr. Casey's comments about communication between regulators and stakeholders; information needs should be characterized in terms more specific than "screening," "hazard identification," or "risk assessment." This is also true for AOP development. Communication needs to go both ways; regulators can also learn from companies about how they gather information to evaluate products and decide which test to conduct. Training is critical for acceptance, especially hands-on training. Multi-use case studies are particularly valuable, and all users should share experiences in forums such as OECD. PCRM fully supports efforts to demonstrate the limitations of in vivo data. Ms. Sullivan noted that the National Academies has convened a panel[31] at the request of EPA to examine use of animal data and wondered if something similar could be convened to address use of animal data in the pharma sector. She also suggested that the FDA's National Center for Toxicological Research be modernized as a center for qualification of new tools, working in coordination with NICEATM. Such a group would have the advantage of working with FDA internal data as well as being able to engage with groups such as the U.S. Pharmacopeia. Referring to the findings of the ICCVAM Metrics Workgroup, she asserted that, while there are limitations to the approach, there is still value in tracking animal use. By tracking animal use over time, factors influencing the choice to use animals can be identified. She encouraged this group to coordinate agencies' efforts to produce status reports so that agencies can learn from each other and develop best practices in reporting to the public.

In follow-up clarifying comments, Dr. Tal noted that there are some circumstances under which the numbers of animals used for testing might be available to agencies. Ms. Sullivan agreed but acknowledged the Metric Workgroup's position that data on animal use is not available to all agencies. Dr. Casey reiterated that the key recommendation from the Metric Workgroup was that each agency has to develop its own metrics, because each agency has a unique set of information available. The important thing now is to understand how agencies are going to use that information to inform stakeholders about progress.

Ms. Laura Alvarez, representing Cruelty Free International, noted that one of the goals in the strategic roadmap is to encourage use of validated methods. Cruelty Free International compiled a list of 10 animal tests that can be replaced now with available alternatives. These were identified at presentations at last year's SACATM meeting, at the 2021 ICCVAM Public Forum, and at the recent World Congress on Alternatives and Animal Use in the Life Sciences. Barriers include lack of global harmonization (key for vaccine batch testing), lack of regulatory enforcement, requirement for product-specific validation, and availability of the alternative or of contract testing facilities that offer the alternative. Cruelty Free International has compiled some suggestions for ICCVAM activities to address implementation of alternatives to biologicals batch testing, antibody production, pyrogenicity testing, and marine biotoxin testing, which are listed in their presentation. Ms. Alvarez closed by expressing concern that the United States might be holding back international efforts to move beyond the guinea pig test for skin sensitization testing by rejection of an OECD proposal to delete the guinea pig test guideline and by the FDA's guidance stating a preference for the guinea pig test over

[31] Information at https://www.nationalacademies.org/our-work/variability-and-relevance-of-current-laboratory-mammalian-toxicity-tests-and-expectations-for-new-approach-methods--nams--for-use-in-human-health-risk-assessment.

the mouse local lymph node assay. Responding to this point, Dr. Kleinstreuer noted that the OECD guideline for in vivo skin sensitization tests has been revised to state that testers should considers all available alternatives, including defined approaches, before running animal tests. Dr. Lowit added that deleting a guideline could cause additional testing because tests run using the deleted guideline may no longer be acceptable for regulatory use. Defined approaches perform well but don't completely cover the chemical space occupied by regulated chemicals, so animal tests are still needed to cover these chemicals. Ms. Alvarez responded that this perspective is helpful and noted that Cruelty Free International is currently interacting with specific agencies on this topic.

Ms. Katrinak, HSUS/HSLF, encouraged consideration of IATAs as representing a more mechanistic representation of chemical effects on humans. Training focused on AOPs will help regulators better understand how chemical interaction with biological pathways affects risk. The EPA policy on skin sensitization is an example of regulatory guidance that provides the clarity that industry needs to confidently utilize NAMs. OECD Guideline 497 is also an important advancement to that end, and ICCVAM agencies should quickly act to update guidance to reflect their acceptance of data from the defined approaches outlined in this document. Ms. Katrinak echoed Ms. Sullivan's comments welcoming the EPA-sponsored National Academies panel and encouraging other agencies to engage in such efforts. She also praised the EPA's retrospective analyses to support establishment of waiver criteria. In addition to identifying opportunities to reduce animal use, such studies also identify animal tests that do not add value to risk assessments and therefore do not need non-animal alternatives developed. She encouraged agencies to support development of animal-based microphysiological systems to increase confidence in their use and replace animal studies for veterinary drug testing and ecotoxicity studies. She closed by encouraging all ICCVAM member agencies to dedicate funding to NAMs development, pursue global acceptance of NAMs, and establish timelines for replacement of animal methods.

Comments from Designated SACATM Discussants

Discussants for "Evolving Approaches to Validation" were asked to consider the following questions:

- What do you think are important aspects that should be considered when updating the existing ICCVAM validation guidelines?
- Do you have any suggestions on how best to support international harmonization while also integrating improvements to accelerate the process of achieving such harmonization?
- How can the biological understanding of chemical effects be better incorporated into NAMs to establish scientific confidence in their routine use?

Dr. Szczepan Baran, Novartis Institute for BioMedical Research, first discussant, noted that education and training is key for advancing acceptance of NAMs, both for students and experienced scientists. Regarding the first two questions, he felt that there needs to be agreement among regulatory agencies and industries on principles and procedures for how to assess performance and build confidence in new methods. Harmonized nomenclature helps with engagement among stakeholders. Guidelines for validation need to be flexible to accommodate advances in technology. Characterization of

reference data could be aided by development of a translational index that could help establish clinical relevance of methods. Use of emerging technologies like digital endpoints could help obtain more information from ongoing studies and will also support development of new models that are clinically relevant. To develop appropriate metrics, it is important to know how NAMs are being utilized and how they're impacting the decision-making process. Agencies need to help technology providers understand the regulatory framework, especially in the context of specific industries. Applicable to all of these is engaging the regulatory agency reviewers because these are the people who are interpreting the data. He wondered whether the FDA's Innovative Science and Technology Approaches for New Drugs pilot program[32] could serve as a model for how to expedite the consideration of new methods by OECD. Because commerce is global, it is important to think globally about how to use new methods. Collaboration is key to addressing all the questions that are being raised.

Dr. Charest, second discussant, noted the progress made in advancing NAMs and the coordination that has been central to this. One aspect to consider when updating the ICCVAM guidance is the role of human biological relevance in the validation or qualification of a method. This is the goal that we should be pursing rather than correlating with an existing animal model. In addition to making biological sense, it is becoming easier to make these connections through the use of human induced pluripotent stem cells and tissues and platforms such as microphysiological systems. Another important element is documenting protocols; more and more methods depend on computational techniques, and documenting these methods is very important. Sometimes these techniques are not entirely transparent, and elements such as software versions can affect outcomes. Regarding regulatory acceptance, he wondered whether results of an evaluation from a test method could be shared across agencies to support harmonization and increase transparency. On an international level, availability of data in public repositories is improving transparency of methods and findings. This should be supported and incentivized as much as possible as it allows data to be used for retrospective studies or leveraged for prospective work. Availability of data also helps address the problem of language barriers. He agreed with the importance of allowing each agency to develop its own metrics, because this allows for accommodation of unique regulatory contexts. These include situations where companies have to work internationally and address requirements imposed by other countries. He echoed previous comments on the need for training, which should include identifying, highlighting, and documenting the shortcomings of the existing animal methods. Compiling and sharing this information could support development of alternatives by highlighting where improvements are needed. Review of a NAM should consider both the biological effect to be predicted and the biological effect to be measured. These aren't always the same thing but a better understanding of both will enable design of NAMs where the two are as close as possible. Identification of valid human biomarkers and incorporating those into NAMs will help build confidence in these approaches.

Dr. Berg, third discussant, expressed strong support for efforts to disengage the use of animal data to validate tests used for human health effects. Validation needs to focus on

[32] Information available at https://www.fda.gov/drugs/drug-development-tool-ddt-qualification-programs/innovative-science-and-technology-approaches-new-drugs-istand-pilot-program.

more human-relevant approaches and expanded to measure effects specific to people who might have underlying health conditions or be experiencing multiple chemical exposures. Studies to better understand mechanisms of human toxicity and human exposure should take these factors into account to better address the needs of vulnerable populations. An international leadership group could facilitate this and support harmonization. Such a group could also facilitate collection of human data to validate NAMs. Open science will eliminate a lot of rework and waste, and the CATMoS project is an example of how this can work. Better communication is needed to clarify the science around both animal-based and alternative methods for non-experts, particularly around specific limitations and applicability domains. She closed by stating that NAMs development should move away from one-to-one replacements and toward aligning with IATAs and AOPs, which will better connect NAMs to human outcomes.

Additional SACATM Comments

Dr. De Abrew commented that care needs to be taken in the case of using data from a single NAM in a hazard context. Also, specificity needs to be considered as much as sensitivity when considering performance. There is a danger in developing a method that generates a lot of false positives.

Dr. Page felt that it's important to define the applicability domain of a method as a way of supporting transparency and an understanding of the specific questions the NAM is meant to address. She called on ICCVAM to identify what works well in interactions with OECD and utilize this with an aim for achieving international harmonization. Conversely, ICCVAM should also identify OECD best practices that could be applied to harmonize practices across U.S. federal agencies. Publication of AOPs will not only help with development of IATAs but will also support a better understanding of chemical effects to guide NAM usage. The concerns that have been expressed about stakeholders not being aware of the existence of NAMs speaks to the need for both education and training and for outreach to understand why accepted NAMs are not being applied. ICCVAM can be a forum to accomplish this. Regarding metrics, ICCVAM needs to hold agencies accountable for communicating their accomplishments and highlighting case studies. She agreed with Dr. Berg's comments about the need for transparency in work being done to replace existing regulatory requirements and wondered how to broaden participation in such discussions to speed both method development and acceptance.

Dr. Clippinger appreciated ongoing efforts to characterize the reproducibility (or lack thereof) in animal methods. She noted NICEATM's analysis of the variability of the rabbit skin assays[33], in which they found lack of reproducibility of categorization of mild to moderate irritants. Consideration of the relevance and mechanism of a NAM is as important as comparing it to the animal test. Language is important here; rather than saying that the NAM is producing the "wrong" results, the reason for the discrepancy should be examined with the goal of accepting the results produced by the more reliable and relevant method. Agencies reviewing NAMs should not automatically discard a method that doesn't reproduce the results of the animal test. The goal of establishing metrics should be to identify where NAMs exist but are not used or areas where NAMs need to be developed, as an aid to prioritizing efforts. She cited as an example a recent PSCI study that identified specific agency concerns surrounding existing NAMs for eye

[33] Rooney et al. 2021. Regul Toxicol Pharmacol. https://doi.org/10.1016/j.yrtph.2021.104920.

irritation testing that can now be addressed. The metrics can be different for each agency as long as they exist and serve to support those goals.

Ms. Leary encouraged those involved in the revision of the ICCVAM validation document to carefully consider the use of the word "confidence". It's important to be clear what is meant by this and specifically that it not simply refer to reproduction of animal data. The goal should be to demonstrate relevance of the new methods, which can be a clearer and more objective goal.

Dr. Tal noted that a combination of concentration-response and time-course experiments are currently being used to build hypotheses that fit NAMs into AOPs. These approaches could be enhanced by gene editing experiments to validate molecular initiating events and key events. These could also help address toxicity of mixtures. She wondered if OECD or other organizations had any ongoing activities around tracking the success rate of NAM development efforts funded by grants. Responding to the last comment, Dr. Kleinstreuer noted that NIEHS has a robust small business grants program, and NICEATM works with the funding office to direct their requests for proposals to spaces where there are needs. ICCVAM members are also active in validation management groups for these grants.

Dr. Kleinstreuer asked Dr. Baran to clarify his idea of a translational index, specifically whether this would be a measure of the impact of a NAM and how he would envision it being used. Dr. Baran replied that he envisioned these being developed for both in vitro and in vivo methods. They would involve collecting data on chemicals tested using a specific method and evaluating whether subsequent clinical research or epidemiological data confirm the effects predicted by that method, which would help validate the use case of the method.

X. Update on NICEATM Computational Resources

Dr. Kleinstreuer noted how valuable feedback from SACATM has been in helping NICEATM update its computational resources. NICEATM's goal is to enable a broad audience of stakeholders to use these tools to engage with alternatives. The most recent publication on ICE[34] is open access and will be linked to the ICE website soon. ICE is designed for ease of use and upholds FAIR (findability, accessibility, interoperability, and reusability) and TRUST (transparency, responsibility, user focus, sustainability, and technology) principles. For example, ICE assays are mapped to both regulatory endpoints and mechanistic targets using controlled vocabularies and ontologies that support integration with other data resources and tools. ICE also includes curated chemical lists and computational workflows.

Dr. Kleinstreuer reviewed the types of experimental data and in silico predictions available in ICE. ICE tools that have been updated recently include Search, Curve Surfer, Physiologically Based Pharmacokinetics (PBPK), IVIVE, and Chemical Characterization. Collaborations with EPA have allowed NICEATM to include consumer use data in the Chemical Characterization tool outputs and working with the developers of the EPA's httk R package has been vital to the development of the PBPK and IVIVE tools. The new Chemical Quest tool was developed specifically in response to a

[34] Abedini et al. 2021. Comput Toxicol. https://doi.org/10.1016/j.comtox.2021.100184.

SACATM request. Dr. Kleinstreuer reviewed other ongoing and completed improvements in response to SACATM requests and feedback, as well as features of the mid-October version 3.5 release and future releases. These include help videos to walk users through query building; support for a variety of chemical identifiers; interactive visualizations of query results; and filtering of specific results to send to other ICE tools or external databases. In addition to feedback from SACATM, NICEATM has a user group within NIEHS that provides useful input, and NICEATM also receives input from ICCVAM members and agency scientists. Dr. Kleinstreuer closed by describing planned efforts to incorporate population variability into ICE tools. ICE already includes virtual population simulations to define confidence intervals around equivalent administered doses and internal concentrations. The next step will be to incorporate influences such as genetic polymorphisms in metabolic enzymes. A partnership with the European Food Safety Authority is being established to help NICEATM to integrate genetically based metabolic variability into the ICE IVIVE and PBPK tools.

Clarifying questions and comments: In response to a question from Dr. Fourches, Dr. Kleinstreuer described data sets that will be soon added to ICE, including in vivo development toxicity data, human skin sensitization data, acute inhalation toxicity data, and CaCo2 permeability data. Dr. Berg asked whether it would be possible to include consideration of environmental influences or comorbidities into ICE simulations of population variability. Dr. Kleinstreuer agreed that would be ideal. In addition to the genetic variability studies, NICEATM is currently running metabolism simulations for 800,000 chemicals in the EPA chemical library to better understand chemical metabolism and refine the confidence intervals generated by ICE predictive tools. The population variability information currently used in ICE may account for some comorbidities. Accounting for environmental exposures will be more complicated and could involve bringing in more epidemiological data. Dr. Sura asked what the most appropriate stage in chemical development would be to apply ICE tools. Dr. Kleinstreuer responded that it would depend on the context. Regulators could use the tools to define a context and framework for risk assessment, or to identify, combine, and analyze data to inform upon regulatory decision making. Method developers might find ICE's chemical lists useful for method development. Chemical companies might find Chemical Quest useful for exploring bioactivity profiles of substances similar to chemicals they are developing.

Public Comments

There were no written comments submitted for this section, nor were there any oral comments presented.

Comments from Designated SACATM Discussants

Discussants for "Update on NICEATM Computational Resources" were asked to consider the following questions:

- What functions or tools in ICE could be improved?
- What functions or tools could be prioritized for future development?
- What other types of data would you like to see in ICE?
- Are there improvements to how data are annotated in ICE that would enhance

interpretation and operability?

Dr. Fourches, first discussant, commented that there have been impressive improvements to ICE over the last year, noting specifically implementation of FAIR principles, improvements to visualizations, and launch of the PBPK tool. He suggested that a direct implementation of a statistical test could be applied to better highlight the difference between a reference chemical and a query chemical. He would also like to see removal of outliers from dose-response curves displayed in Curve Surfer and subsequent recalculating of the activity metric. Improvements in portability of ICE components could support wider use by industry. Chemical Quest is a great resource that will be improved by the availability of new molecular descriptors. Suggestions for data to be added to ICE included proteomics or metabolomics data that could reveal metabolic profiles in response to chemical exposure. Those data are available elsewhere but it would be useful to have them in the context of other ICE data. Continued implementation of FAIR and TRUST standards will help interpretation and operability, as will improvements in download formatting and making sure the most up-to-date file format is used.

Dr. Sura, second discussant, noted that ICE has tremendous potential for both regulators and industry. It is easy to use and very helpful for evaluating novel substances. She noted the improvements to graphics. Users who are not toxicologists or familiar with the regulatory context might be helped by the availability of a categorical characterization of a new chemical, and more context for assay results. Another useful feature would be side-by-side characterization of two chemicals for specific endpoints such as genotoxicity or skin sensitization. She also expressed interest in including hepatotoxicity as an endpoint of interest. In general, she would like to see features that would better support chemical companies trying to develop safer chemicals, and more information on hazard characterization at human-relevant exposures. She closed by stating she looked forward to the availability of training videos but added that targeted training sessions would be particularly helpful.

Additional SACATM Comments

Dr. Kleinstreuer responded to several points made by the discussants. She emphasized the potential drawbacks of including flags for potential hazard in query results but agreed that side-by-side comparisons of chemical profiles could be useful, as could identifying reference compounds for particular targets. There's a Tox21 cross-partner project ongoing in that area right now, and perhaps outcomes of that project could be applied to ICE. Adding statistical tests is a good idea. She clarified that ICE input data from EPA are already curated to remove outliers, but it might be possible to add annotations to Curve Surfer to clarify that. Some of ICE tools are already portable, but NICEATM will explore how to broaden this capability where it would be most useful.

Dr. De Abrew asked if Dr. Kleinstreuer could provide examples of a regulatory authority or industry using the tool for decision making or submission, and if so, whether they could be shared as case studies. Dr. Kleinstreuer referred to the DoD project described in her comments the previous day in which an additivity formula and ICE data were applied to predict toxicity of mixtures. Communications to NICEATM from other ICCVAM member agencies have indicated that they are using ICE internally; NICEATM does not have details of the specific applications, and NICEATM would welcome descriptions of

these from the agencies. NICEATM does collect general data on access to ICE via Google Analytics, and Dr. Kleinstreuer said she would incorporate these data into future presentations to highlight ICE's impact.

XI. Adjournment

Dr. De Abrew invited concluding remarks from SACATM members.

- Ms. Leary complemented NICEATM on advancements in ICE.
- Dr. Page noted that, while most of the discussion centered around what remains to be done, she felt that it was important to acknowledge what the ICCVAM agencies have been able to achieve and to encourage continued progress.
- Dr. Ushio appreciated the comments regarding moving away from animal tests as a reference standard for validation.
- Dr. Fourches noted the advances that have been made toward acceptance of predictive models and their growing importance in decision making. This is a sign of the maturity of this technology that has been well represented in the presentations in this meeting.

Dr. Brian Berridge, NIEHS, thanked the committee for their engagement and comments. In addition to moving away from dependance of animals, the work represented in this meeting is supporting a better representation of human outcomes. The interdisciplinary nature of this advisory group is an important element of that success.

Dr. Scruggs thanked all participants and support staff and noted the important contributions made by Dr. Elizabeth Maull (recently retired from NIEHS) in the planning of this meeting. Slides from the meeting will be made available on the NTP website when they meet government accessibility guidelines, and attendees will be notified when slides and minutes are available.

Dr. De Abrew adjourned the meeting at 3:38 p.m.

K. Nadira De Abrew, PhD

SACATM Chair

Date: 01/27/22