Summary Minutes

Scientific Advisory Committee on Alternative Toxicological Methods Meeting
September 19-20, 2019
Crowne Plaza Crystal City
Arlington, VA

CONTENTS

I. Location of Background Materials and Presentations

Background materials and presentations for the 2019 Scientific Advisory Committee on Alternative Toxicological Methods meeting are available on the National Toxicology Program Past SACATM Meetings page (https://ntp.niehs.nih.gov/events/past/index.html?type=SACATM)

II. Frequently Used Abbreviations

ADME	absorption, distribution, metabolism, and excretion
AOP	adverse outcome pathway
API	American Petroleum Institute
CATMoS	Collaborative Acute Toxicity Modeling Suite
CPDat	Chemicals and Products Database (EPA)
CV	cardiovascular
DARPA	Defense Advanced Research Projects Agency (DoD)
DoD	U.S. Department of Defense
EPA	U.S. Environmental Protection Agency
ESAC	EURL ECVAM Scientific Advisory Committee
EURL ECVAM	European Union Reference Laboratory for Alternatives to Animal Testing
FAIR	findability, accessibility, interoperability, and reusability of data
FDA	U.S. Food and Drug Administration
GUI	graphical user interface
HSUS	Humane Society of the United States
httk	high throughput toxicokinetics
IATA	integrated approach to testing and assessment
ICATM	International Cooperation on Alternative Test Methods
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ICE	Integrated Chemical Environment
ILS	Integrated Laboratory Systems, Inc.
IVIVE	in vitro to in vivo extrapolation
MPS	microphysiological systems
NAMs	new approach methodologies
NCATS	National Center for Advancing Translational Sciences (NIH)

NICEATM	NTP Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences (NIH)
NIH	National Institutes of Health
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OPERA	Open Structure-activity/property Relationship App
OPP	Office of Pesticide Programs (EPA)
ORD	Office of Research and Development (EPA)
PCRM	Physicians Committee for Responsible Medicine
PISC	PETA International Science Consortium, Ltd.
QSAR	quantitative structure-activity relationship
SACATM	Scientific Advisory Committee on Alternative Toxicological Methods

III. Attendance

The Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) met on September 19 and 20, 2019, at the Crowne Plaza Crystal City hotel, Arlington, Virginia. The following individuals attended the meeting:

SACATM Members

Michael Bolger, PhD, Simulations Plus, Inc.

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

Amy Clippinger, PhD, PETA International Science Consortium, Ltd.

Kelly Coleman, PhD, DABT, RAC, Medtronic PLC

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

Sean Gehen, PhD, DABT, Corteva Agriscience™

Hisham Hamadeh, PhD, DABT, MBA, Genmab US, Inc.

Lawrence Milchak, PhD, DABT, 3M

Pamela Spencer, PhD, DABT, ANGUS Chemical Company (chair)

ClarLynda Williams-Devane, PhD, Fisk University

Hao Zhu, PhD, Rutgers University at Camden

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

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

John Elliott, PhD, National Institute of Standards and Technology (NIST)

Suzanne Fitzpatrick, PhD, U.S. Food and Drug Administration (FDA)

John Gordon, PhD, U.S. Consumer Product Safety Commission (CPSC)

Bert Hakkinen, PhD, National Library of Medicine

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

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

Seila Selimovic, PhD, National Institutes of Health (NIH)

Other ICCVAM Representatives

Warren Casey, PhD, DABT, NIEHS

Jennifer Goode, FDA

Eric Hooker, MS, CPSC

LTC Matthew Johnson, DoD

Nicole Kleinstreuer, PhD, NIEHS

Geoff Patton, PhD, FDA

Monique Perron, PhD, U.S. Environmental Protection Agency (EPA)

International Cooperation on Alternative Test Methods Representatives

Takao Ashikaga, PhD, Japanese Center for the Validation of Alternative Methods

Joao Barroso, PhD, European Union Reference Laboratory for Alternatives to Animal Testing

Charu Chandrasekara, PhD, Canadian Centre for the Validation of Alternative Methods

Tae Sung Kim, PhD, Korean Center for Validation of Alternative Methods

National Institute of Environmental Health Sciences Staff

Linda Birnbaum, PhD, DABT, ATS

Jed Bullock, MPA

Robbin Guy

John Maruca (Image Associates, NIEHS support contractor)

Elizabeth Maull, PhD, Designated Federal Official

Nathan Mitchiner (NETE, NIEHS support contractor)

Lingamanaidu Ravichandran, PhD

Integrated Laboratory Systems, Inc. (NICEATM support contractor) Staff

David Allen, PhD

Ella Darden

Kamel Mansouri, PhD

Steven Morefield, MD

Catherine Sprankle, MS

Public

Emily Anderson, MS, Physicians Committee for Responsible Medicine (PCRM)

Manjula Aysola, MS, MilliporeSigma

Szczepan Baran, VMD, Novartis

Richard Becker, PhD, American Chemistry Council

Bob Diderich, Organisation for Economic Co-operation and Development (OECD)

Carol Eisenmann, PhD, Personal Care Products Council

Anne Gourmelon, MS, OECD

Esther Haugabrooks, PhD, PCRM

Gina Hilton, PhD, People for the Ethical Treatment of Animals

Kristin Isaacs, PhD, EPA

Catharine Krebs

Sue Leary, MS, Alternatives Research and Development Foundation

Robert Leverette, PhD, RAI Services Company

Lucie Low, PhD, National Center for Advancing Translational Sciences (NCATS)

Elizabeth Margosches, PhD

Jean Orelien, PhD, ScitoVation

Pat Rizzuto, Bloomberg Environment

Jessica Ryman-Rasmussen, PhD, DABT, American Petroleum Institute

Joshua Schmidt, PhD, SenzaGen

Danilo Tagle, PhD, NCATS

Alexandra Turley, PhD, FDA

Nina Wertan, MPA, The Humane Society of the United States

September 19, 2019

IV. Welcome and Opening Remarks

The National Toxicology Program (NTP) convened the 2019 meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) on September 19. Dr. Pamela Spencer, ANGUS Chemical Company, SACATM chair, called the meeting to order at 9:00 a.m., welcomed everyone to the meeting, and asked attendees to introduce themselves. Dr. Elizabeth Maull, the SACATM Designated Federal Official, read the conflict of interest statement and reviewed meeting logistics. Dr. Linda Birnbaum, National Institute of Environmental Health Sciences (NIEHS) and National Toxicology Program (NTP) Director, provided opening remarks, introduced international attendees, and presented departing SACATM members with certificates of appreciation. Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) co-chair Dr. Emily Reinke, U.S. Department of Defense (DoD), and Dr. Warren Casey, Director, NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) added their welcomes to the committee. Drs. Reinke and Casey recognized Dr. Birnbaum on her retirement for her support of ICCVAM during her tenure as NIEHS Director.

V. US Strategic Roadmap: New Approaches to Validation

Overview and U.S. Activities

In introducing the first sessions, Dr. Casey reflected on recent progress towards replacement of animal testing, citing the U.S. Food and Drug Administration (FDA) Predictive Toxicology Roadmap[1] and the September 10 U.S. Environmental Protection Agency (EPA) directive to phase out mammalian testing[2] as examples of agency mandates needed to effect change. Dr. Casey further commented on agency collaborations that facilitated acceptance of new methods, citing FDA's recent efforts with industry and standards organizations to validate human skin models for medical device testing and validation of the electrophilic allergen screening assay by three federal laboratories.

Persistent challenges to progress include lack of international adoption of test methods accepted in the U.S. and the amount of time required for acceptance by the Organisation for Economic Co-operation and Development (OECD) and the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use. Interactions with these organizations are key to addressing these challenges and present opportunities to reduce animal use in non-regulatory areas of testing, such as antibody production. Dr. Casey challenged the audience to consider new approaches for method validation.

[1] Available at https://www.fda.gov/science-research/about-science-research-fda/fdas-predictive-toxicology-roadmap

[2] Available at https://www.epa.gov/newsreleases/administrator-wheeler-signs-memo-reduce-animal-testing-awards-425-million-advance

Evaluation of a Proposed Approach to Refine the Inhalation Risk Assessment for Point of Contact Toxicity: A Case Study Using a New Approach Methodology

In introducing the first talk, Dr. Casey cited an EPA collaboration with Syngenta as an example of a new validation approach. Dr. Monique Perron, EPA, presented a validation case study for a new approach methodology (NAM) for inhalation risk assessment, which traditionally relies on in vivo repeat-dose toxicology studies. NAMs, aimed at avoiding these studies, provide an opportunity for more human-relevant testing approaches based on both the differences between the rat and human respiratory systems and the limitations of the animal-based approaches in identifying respiratory irritants.

This project, initiated by Syngenta, proposed a 3D in vitro model for deriving a point of departure for the respiratory irritant chlorothalonil and led to an open collaboration between the EPA Office of Pesticide Programs (OPP) and Syngenta, and involved NICEATM and the EPA Office of Pollution Prevention and Toxics. Based on the biological mechanism and progression of toxicity for chlorothalonil, the MucilAir™ system was identified as the most relevant in vitro model. A point of departure was identified and related to human exposure through computational fluid dynamic modeling that incorporated human-relevant particle size distributions.

Panelists at a 2018 Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel meeting[3] agreed that there was no support for using an animal study in this context. Other recommendations included using the most sensitive endpoint, incorporating repeat dosing within vitro assays, and basing particle size distributions on empirical data. Syngenta, OPP, and other stakeholders are addressing these issues.

Dr. Perron noted other ongoing research projects with inhalation testing alternatives that EPA has been involved in:

- A proof of concept study by EPA's Office of Research and Development comparing commercially available 2D and 3D models using chemicals nominated by the EPA Office of Chemical Safety and Pollution Prevention.
- Participation on an NIEHS Phase 2B grant steering committee for the validation of a human airway epithelial model for acute toxicity.
- Collaboration with PETA International Science Consortium, Ltd. (PISC) to investigate in vitro systems with a phased approach.
- Sponsorship of a Society of Toxicology ancillary meeting on the state of the science for inhalation alternatives.

Dr. Perron commented that the Syngenta case study illustrates how strategic roadmap principles can guide activities. While companies hesitate to adopt NAMs due to the perception that agencies will not

[3] Meeting materials are available at https://www.epa.gov/sap/meeting-materials-december-4-6-7-2018-scientific-advisory-panel-0

accept them, last week's announcement calling for a reduction in animal use by 2035 is a signal that EPA is moving towards greater acceptance of NAMs. While this study was well-received, it took years to complete, calling for a need for more rapid advancement to achieve EPA's goal.

Clarifying questions and comments:

In response to a question from Dr. Lawrence Milchak, 3M Medical Department, Dr. Perron indicated that the case study evaluated direct liquid exposures, which could be considered a worst-case scenario because the entire epithelial surface is covered. Ongoing ORD studies are modeling aerosol exposures. Dr. Coleman asked if the Lautenberg Chemical Safety Act set the stage for last week's EPA directive. Dr. Perron responded that she wasn't aware of a direct connection, but the directive does mention the act. The large amount of data required by OPP may also have been a factor. Dr. Coleman commented that, as with the European Cosmetics Directive, it could be a case for creating a deadline to drive progress. Dr. Amy Clippinger, PISC, asked if there was a timeline for either the ORD testing or an updated risk assessment for chlorothalonil. Dr. Perron responded that while she was unaware of the ORD timeline for testing, the risk assessment is scheduled to be issued in FY 2020 as part of its registration review. In response to a question from Dr. Joseph Charest, Charles Stark Draper Laboratory, Inc., Dr. Perron commented that MucilAir was the only in vitro system tested for the case study. Syngenta's evaluation indicated that, of the models available at the time, it was the one best suited to address their research question.

Public Comments

Written Public Comments

Two written public comments were submitted on behalf of the American Petroleum Institute (API) and the Humane Society of the United States (HSUS) for this section.[4]

Oral Public Comments

Dr. Jessica Ryman-Rasmussen, API, noted that API considers adverse outcome pathways (AOPs) to be an important element in the implementation of NAMs. She presented suggestions for increasing the utility and uptake of AOPs by the regulatory and regulated communities, which are discussed in more detail in API's written comments.

1. An OECD recommendation on mutual acceptance of AOPs may be needed to increase the utility and uptake of AOPs in regulatory contexts across countries.
2. AOPs based on known mechanisms of toxicity would facilitate their acceptance in regulatory contexts.
3. While key characteristics are appropriate for data organization or weight-of-evidence assessments, they should not be equated with or used as substitutes for AOPs nor should they

[4] Written public comments for all topics are available at https://ntp.niehs.nih.gov/go/meeting; select Event Type "SACATM" and click on "Meeting Materials."

represent stand-alone approaches for hazard identification or classification.

4. Utilization of AOPs may be limited by the applicability domain of the NAMs used to support them. NAMs used to support AOPs should encompass substances with a broad range of physicochemical properties, in particular complex mixtures of hydrophobic substances.

Dr. Kristie Sullivan[5], representing the Physicians Committee for Responsible Medicine (PCRM), hoped that the success of the EPA-Syngenta project would encourage broader consideration of 3D respiratory models by regulated industry stakeholders. EPA is providing transparency and clarity to the regulated community regarding acceptance of alternative approaches. Dr. Sullivan requested that EPA share more information about in vitro approaches proposed by industry as well as issue more frequent updates on waivers granted.

Ms. Nina Wertan, representing HSUS, urged ICCVAM to encourage agencies to increase communication and engagement on NAMs. Agencies should keep their regulated industries apprised of their activities related to the strategic roadmap. Ms. Wertan cited the recent FDA assessment of dog studies for testing of food and color additives as a good example; HSUS looks forward to FDA communicating the conclusion of this study to the regulated industry. Communication of agency activities supports confidence that the elimination of animal testing does not compromise human and environmental safety. Ms. Wertan commended EPA on their recent announcement and encouraged other agencies to follow suit. To support international harmonization, HSUS strongly encouraged agencies to engage with the International Cooperation on Alternative Test Methods (ICATM), which has fostered acceptance of non-animal approaches for skin sensitization testing. Furthermore, she recommended that ICCVAM agencies increase their engagement with OECD expert groups. To support development of NAMs, grant review criteria should be modified to include specific criteria pertaining to the development and use of NAMs, and the National Institutes of Health (NIH) should issue more grant opportunities for NAMs similar to those issued through NIEHS small business innovation research grant programs.

Dr. Richard Becker, representing American Chemistry Council, noted that the private sector sees great value in government and private sector investment in NAMs. Partnerships among industry, agencies, and the animal welfare sector enables advancement of private sector work to a broader sphere. While one-to-one replacements are not feasible to replace whole-animal testing, NAMs are more likely to be useful as parts of integrated approaches to testing and assessment (IATAs) based on AOPs that move from computational evaluations to higher complexity tests.

Clarifying questions and comments:

In response to a question posed by Dr. John Elliott, National Institute of Standards and Technology, Dr. Ryman-Rasmussen responded that she was unaware of any specific examples of assays that have succeeded with hydrophobic substances. However, Concawe, a European petroleum industry

[5] Dr. Sullivan presented her remarks via telephone.

consortium, is currently exploring this in their Cat-App project[6]. API considered computational approaches to be more useful.

Comments from Designated SACATM Discussants

Dr. Amy Clippinger and Dr. Sean Gehen, Corteva Agriscience, were the discussants for the initial presentations. Dr. Clippinger characterized EPA's recent activities as examples of how agencies can encourage regulated industry to develop and use alternatives. Dr. Gehen added that the common need to address the uncertainty around inhalation exposure given that the rodent model is not particularly human relevant motivated the collaboration between EPA and Syngenta. To facilitate more partnerships, Dr. Clippinger called for involvement of more agencies and the inclusion of non-federal scientists within the ICCVAM work groups. While SACATM is a useful venue for discussions, more frequent meetings or increased engagement of members would be worthwhile.

Both reviewers commented on the need for transparency in keeping the public informed about how new methods are developed and reviewed to build confidence in the process. EPA's transparency about their decisions and the science used to support them provided stakeholders with an example of how new methods are vetted. In addition, Dr. Gehen stated that building industry confidence that new methods will be acceptable to regulators will also encourage adoption of new methods.

Dr. Gehen stated that a way to lower the barrier to adoption and increase investment in new approaches is needed and recommended deconstructing the complexity of a biological system to identify and implement more human relevant endpoints and assays.

The reviewer's recommendations included expanding beyond the portal of entry toxicity approach to assess systemic toxicity of inhaled substances and ensuring that the development of new methods adequately addresses the diversity of chemical space. Dr. Clippinger encouraged agencies to incentivize development and implementation of new approaches with, perhaps, an expedited review. She also noted that training for regulators is needed to facilitate consistency in new method acceptance.

Response to SACATM Comments

Addressing Dr. Clippinger's comments, Dr. Casey indicated his supported of more frequent SACATM interactions and will explore opportunities to comply with the suggestion. However, federal rules and regulations make the inclusion of nonfederal scientists on ICCVAM workgroups challenging as agencies are precluded from open discussions in forums that are not considered public. Dr. John Gordon, US Consumer Product Safety Commission, added that the Sunshine Act prohibits agencies from participating in nonpublic meetings with nongovernment stakeholders. Dr. Casey suggested that stakeholders initiate a group and invite appropriate federal agency scientists to participate.

In support of transparency, Dr. Casey commented that the agencies, particularly the regulatory agencies, need webpages that explicitly and specifically state what tests the agency will accept, how they will accept them, and what they will accept them for.

[6] Information available at https://www.concawe.eu/cat-app/

Dr. Spencer agreed that we need a quicker, more efficient path to completion for these partnerships to work.

International Activities

OECD Activities to Increase the Utility and Uptake of AOPs in Regulatory Contexts Across Countries

Mr. Bob Diderich, OECD, indicated that the evolution of regulatory agency requirements away from "test results" and toward "information needs" complicates OECD's primary goal of achieve international harmonization for their chemical safety testing program. To help address this, OECD has published guidance[7] on how to use AOPs to promote a common understanding of IATA use. They have also compiled case studies that support the use of IATAs for regulatory decision-making and developed guidance documents to support greater harmonization.

While stating that there are few endpoints for which AOPs are completely defined, Mr. Diderich added that incomplete AOPs are still useful. AOPs allow testing of hypotheses generated by read-across evaluations, the most commonly used non-animal toxicity assessment approach, and determination of whether the chemical of interest works through the same mechanism as a related chemical, even for complex endpoints such as developmental toxicity. Tools within OECD's QSAR Toolbox facilitate read-across evaluations by allowing the user to group chemicals. AOPs may provide the mechanistic models of toxicity needed to create these groupings.

Mr. Diderich described several projects in which AOPs have or are being used to develop mechanistic understanding and testing strategies. However, to achieve harmonization between countries, defined approaches need to be rule-based with a fixed data interpretation procedure. OECD is working on a defined approach for skin sensitization that combines test results, computer predictions, and an interpretation strategy. While delayed by concerns over the lack of expert judgement, OECD hopes to have the skin sensitization defined approach approved next year. Tests using this approach would fall under the OECD Mutual Acceptance of Data agreement.

OECD also believes that AOPs will help in the interpretation of non-standard in vitro test results. The expectation is that additional mechanistic knowledge will help regulators determine how these tests inform the adverse outcome, which will help with regulatory decision-making. OECD has a guidance document to aid this[8].OECD has an AOP development program, and the number of AOPs being submitted has become so large that they need to prioritize evaluations. Top priority will be given to AOPs that address a regulatory need, have available relevant assays or testing strategies, link to ongoing or future OECD projects, and complement an existing AOP.

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[7] OECD Series on Testing and Assessment No. 260, Guidance Document for the Use of Adverse Outcome Pathways in Developing Integrated Approaches to Testing and Assessment, available at http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2016)67&doclanguage=en

[8] OECD Series on Testing and Assessment No. 211, Guidance Document for Describing Non-guideline In Vitro Test methods, available at http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2014)35&doclanguage=en

Clarifying questions and comments:

Dr. Coleman and Dr. Milchak asked questions about the QSAR tool used in the defined approach for skin sensitization and how the AOP framework fit within to the chemical registration structure, respectively.

Mr. Diderich responded to Dr. Coleman that they are using the OECD QSAR tool for the defined approach, but Lhasa Limited is advocating adding predictions from their software package, Derek Nexus. In response to Dr. Milchak, Mr. Diderich stated that increased AOP knowledge will allow us to identify assays to use for screening, which in turn will allow authorities to ask for information on endpoints early in the registration process.

Antibodies and Non-antibody Affinity Reagents Generated Using Animal-free Technologies, for Use in Research and Diagnostics

Dr. João Barroso, European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM), introduced his topic by noting that the EURL ECVAM Scientific Advisory Committee (ESAC) ESAC reviewed the scientific validity of antibodies and non-antibody affinity reagents generated using animal-free technologies and used in research, diagnostics, and regulatory applications at a 2018 meeting. The review focused on non-animal derived antibodies generated by phage display technology due to the maturity of the technology, the lack of perceived hurdles to acceptance, and the availability of evidence supporting their utility and relevant applications. ESAC recommended, however, that a review of non-antibody affinity reagents as replacements for animal-derived antibodies would be useful.

Dr. Barroso reviewed the advantages of non-animal derived antibodies:

- Control over affinity selection conditions.
- Free choice of detection systems.
- Sequence-defined antibodies.
- Ability to reconstitute antibodies with identical binding and specificity profiles.
- Additional technical properties that improve reproducibility.

Dr. Barroso also corrected some misconceptions about non-animal derived antibodies. Low affinity claims can be addressed through appropriate selection and affinity maturation. Concerns about the lack non-animal derived antibody providers will improve as demand improves. Non-animal derived antibodies are subject to the same limitations as conventional antibodies; these limitations represent opportunities for research.

The ESAC concluded:

- Non-animal derived antibodies are mature reagents generated by a proven technology.
- Non-animal-derived antibodies offer significant additional scientific benefits.

- There is a need to promote accessibility of non-animal derived antibodies within the research, diagnostic, and regulatory communities.

Overall, the ESAC concluded that non-animal derived antibodies can replace animal-derived antibodies in most applications and potentially improve reproducibility. As this recommendation has policy implications, EURL ECVAM is contributing to a JRC Science for Policy report on non-animal derived antibodies. This policy report will recommend raising awareness and disseminating information; education and training; prioritization of non-animal antibody use into project authorization; discourage use of antibodies generation by traditional in vivo methods in newly funded EU projects; and providing funding to characterize existing affinity reagents to build on current knowledge.

Clarifying questions and comments:

Drs. Gehen and Hamadeh asked clarifying questions regarding the availability and uses of non-animal derived antibodies, as well as differences between the animal and non-animal derived products.

Dr. Barroso responded that non-animal derived antibodies are available from Bio-rad and other suppliers. While not as widely available as animal-derived antibodies, custom production is available. Non-animal derived antibodies are also used in diagnostic reagents. Dr. Barroso was not aware of any studies characterizing the differences in neutralizing antibody production from animal and non-animal derived antibodies. While immunogenicity and potency of the non-animal derived antibodies need to be characterized, and one should always use the best reagents available, this is common to both animal and non-animal derived antibodies.

Comments from Designated SACATM Discussants

Discussants for the second set of presentations for New Approaches to Validation were Dr. K. Nadira De Abrew, The Proctor and Gamble Company, Dr. Coleman, Dr. Gehen, and Dr. Milchak. Both Dr. De Abrew and Dr. Gehen indicated that their organizations use AOPs internally. Proctor and Gamble considers MoA/AOPs in addressing specific data gaps in their risk assessments and to develop read-across strategies. Dr. Gehen indicated that Corteva uses OECD-endorsed AOPs conceptually to evaluate the human relevance of, for example, rodent liver tumors. AOPs are also used in research and development to identify compounds with favorable profiles. Corteva is considering an AOP-based tiered approach to evaluate endocrine disruption for internal decision making. Dr. Milchak stated that 3Ms relies on a combination of computational approaches and historical knowledge for inhalation toxicity screening as it is unclear how AOPs fit into the regulatory framework.

All discussants had recommendation for expediting the development and use of AOPs in regulatory decision making. Dr. De Abrew encouraged the use of AOPs to fill specific data gaps and suggested that the regulatory agencies consider how AOPs might be incorporated into larger risk assessments. Dr. Gehen commented that the identification of needs by ICCVAM or OECD could drive specific AOP acceptance and use. Dr. Coleman added that public-private partnerships will facilitate the uptake of AOPs. In Dr. Milchak's opinion clarification of how AOPs fit into the regulatory framework would

facilitate adoption.

To the question regarding how best to promote the findings from the EURLECVAM report as communicated by Joao Barros, Dr. Milchak indicated that international harmonization will promote adoption of non-animal derived affinity reagents by multinational corporation.

Additional SACATM Comments

Dr. Michael Bolger, Simulations Plus, Inc., noted that his company has been looking at modeling liver and kidney injury. There are some in vitro data on drugs to support development of models, but more data on supplements and chemicals are needed to build good QSAR models.

Additional Comments

Dr. Charu Chandrasekara, Canadian Centre for the Validation of Alternative Methods, commented that adoption of non-animal-derived affinity reagents would require a strategic plan and a top down approach. As ICCVAM and SACATM are advocating for non-animal testing approaches, it makes sense for them to have a role in this effort. Furthermore, NIH could drive the change by disincentivizing the use of animal-based antibodies. Production of antibodies is a multibillion-dollar industry with huge fines levied against it related to animal welfare violations.

Dr. Reinke agreed that ICCVAM should have a role in this. It makes sense that if you are implementing non-animal testing approaches you should avoid using animal-derived reagents. NICEATM will circulate the ESAC report to ICCVAM member agencies when available and ICCVAM will engage in activities to raise awareness.

Dr. Gordon suggested that EPA consider expanding its recent directive to include antibody production in animals, and Dr. Perron agreed that this needs to be discussed.

Mr. Diderich noted that currently the primary application for AOPs is as the scientific basis of method selection and development. For AOPs to be applied to risk assessments they will need to be much more quantitative. However, AOPs can help interpret nonstandard information, such as literature derived data, used in risk assessments.

ICCVAM and OECD could assist in the development of AOPs by promoting the AOP development program. Contributions to the AOPwiki is voluntary and without incentives to encourage contributions.

Dr. Casey noted that AOP acceptance was initially hindered by a lack of focus on regulatory endpoints. The utility of partial AOPs to researchers needs to be recognized.

As a final note, Dr. Casey announced that NICEATM has tentatively planned a December workshop on non-animal affinity reagents to ensure that all voices are heard without duplicating the ESAC effort.

VI. New Approach Methodologies: Computational Tools

Balancing Machine Learning and Mechanistic Modeling

Dr. Nicole Kleinstreuer, NICEATM Deputy Director, introduced her topic by describing two

"competing" approaches to modern toxicology and drug discovery: building testing strategies or models exclusively on existing biological knowledge ("expert-driven") and generating as much data as possible and let the machines sort it out ("data-driven"). Success will come from leveraging both approaches, given availability of appropriate resources.

Building predictive models to address human-relevant endpoints requires curated data as inputs to machine learning models. These models can be used to prioritize testing that can better define the models in an iterative process. The ultimate goals are to provide "FAIR" (findable, accessible, interoperable, and reusable) resources and inform regulatory decision making. NIH and the broader scientific community have adopted the FAIR principles to support transparency of scientific work. NICEATM, working with the NIEHS Office of Data Science, is engaged in the construction of NIEHS Data Commons, which will provide a common platform to access NIEHS data, enable curation and annotation of the data, and support interaction between different data sets.

NICEATM's contribution, the Integrated Chemical Environment (ICE),[9] includes data sources such as validation studies, databases, published data, and computational models. NICEATM is working towards obtaining an International Science Council Core Trust Seal for ICE, which is consistent with NICEATM's goals of transparent and robust data access. Dr. Kleinstreuer noted that ICE is the only source of curated Tox21 data that takes into consideration factors such as chemical quality control and curve fit. Data available in ICE include human and animal toxicity data and predictions of physicochemical properties and environmental fate. NICEATM plans to add acute inhalation data, skin and eye irritation data, and toxicokinetic data to support in vitro to in vivo extrapolation (IVIVE) in the future. ICE includes tools for machine learning, chemical characterization, and IVIVE, all provided via a user-friendly graphical user interface (GUI). The IVIVE tool, based on the EPA high throughput toxicokinetics (httk) model, was developed in response to a suggestion from SACATM. New models will come online in ICE as httk evolves. A critical component of IVIVE analyses is the availability of absorption, distribution, metabolism, and excretion (ADME) properties; ICE has prediction models for these properties when experimental data are not available.

Dr. Kleinstreuer reviewed how NICEATM has applied machine learning to endpoints of regulatory importance. Three global collaborative QSAR modeling projects have addressed estrogen receptor pathway activity, androgen receptor pathway activity, and acute oral systemic toxicity. The key to all these models is robust reference data. Dr. Kleinstreuer reviewed the development of the uterotrophic assay database that enabled validation of the estrogen receptor model. This database is now being used as a training set for developing an automated approach to extracting literature data, which is being applied to developmental toxicity studies. Other developmental toxicity projects include reviews of NTP studies and European Chemicals Agency submissions. NICEATM is mapping results to controlled vocabularies and ontologies, which will facilitate integration with other datasets to provide

[9] Available at https://ice.ntp.niehs.nih.gov/

robust training sets for better models.

NTP has established three health effects initiatives in cancer, developmental neurotoxicity, and cardiovascular (CV) toxicity. The CV toxicity effort will develop a mechanistic approach to predicting this toxicity that prioritizes non-animal approaches within an evidence-based testing paradigm. The project has identified mechanistic bases for several different CV failure modes. Some key events, common to several modes, could inform testing strategies to screen for CV risk or support identification of reference chemicals. This approach was successfully used to develop an AOP for embryonic vascular development.

Dr. Kleinstreuer invited SACATM to consider 1) how to leverage modeling techniques to predict toxicity of mixtures across heterogeneous populations and 2) how to build data sets that will support development of models of mixture toxicity for human health endpoints. NICEATM has had some success in building QSAR models to predict ocular hazards of mixtures, specifically differentiating chemicals falling into EPA hazard categories I and II (requiring eye protection when handling) from chemicals falling into hazard categories III and IV (eye protection not required). However, the variability of the in vivo reference test method continues to be a challenge.

Clarifying questions and comments:

SACATM members, Drs. Zhu, De Abrew, Gehen, and Williams-Devane, had clarifying question related to mixtures and the ICE database. In response to the question posed by Dr. De Abrew, Dr. Kleinstreuer indicated that her references to mixtures primarily encompassed formulations with defined components. Ongoing skin sensitization testing at NTP includes a diversity of mixtures to further characterize the applicability domain of the in vitro assays. Dr. John Gordon, CPSC, indicated that the Consumer Product Safety Commission would be interested in working with NICEATM on model development as they have a lot of human data on mixtures. Dr. Kleinstreuer added that NICEATM would need data on the individual components making up the mixture.

Responding to a question from Dr. Zhu, Dr. Kleinstreuer indicated that ICE data used by the httk model are included in the R package and can be downloaded. The models used to predict those parameters can also be shared. Dr. Gehen asked if users can model their own data. Dr. Kleinstreuer replied that ICE tools are currently limited to searching and modeling data within its database, but support of user-provided data is a common request that NICEATM is working on addressing. Dr. ClarLynda Williams-Devane, Fisk University, asked how the ICE machine learning algorithms were chosen and if there is a way for the user to subset their data before building a model. Dr. Kleinstreuer indicated that the ICE machine learning tool is primarily an educational resource; as such, NICEATM chose the most common approaches. Model documentation is found on the website. ICE supports subsetting of data.

Collaborative Acute Toxicity Modeling Suite

Dr. Kamel Mansouri, Integrated Laboratory Systems, Inc. (ILS; contractor supporting NICEATM), reviewed the modeling project behind NICEATM's Collaborative Acute Toxicity Modeling Suite

(CATMoS). The project asked participants to build models to predict five endpoints defined by regulatory agencies. Dr. Mansouri reviewed the data set used for modeling and how data were prepared: the data were standardized, divided into training and evaluation sets so that distributions of chemical classes, toxicity classes, and use categories were maintained, and the training set was provided to the modelers. The evaluation set was used to evaluate the models returned by the participants. A third data set, the prediction set, constituted nearly 50,000 structures from a variety of sources, including all the chemicals in the evaluation set. All participants generated predictions for the prediction set, which were used to develop a consensus model.

Dr. Mansouri reviewed the models submitted, evaluation criteria, and the coverage of the models. Concordance of the models was in general very high. Developing the consensus model was a two-step process in which predictions from all models for each chemical were combined and then subjected to a weight-of-evidence evaluation to identify a winning bin for each prediction. An advantage of a consensus model is that it achieves a broader applicability domain than any single model. Statistics for the consensus model indicate that it predicts toxicity about as well as in vivo data. The consensus model can be used to predict toxicity of new compounds, with predictions based on a weighted nearest-neighbors approach.

NICEATM is working with regulators to develop ways to optimize CATMoS and better display and interpret the predictions, and agencies have provided chemicals for this purpose. CATMoS models are implemented in the OPEn Structure-activity/Property Relationship App (OPERA), which is available in both command-line and GUI implementations. In addition to predicting acute toxicity, OPERA can predict physicochemical properties, ADME properties, environmental fate endpoints, and estrogen and androgen receptor interactivity. CATMoS toxicity predictions will soon be available via ICE and the EPA CompTox Chemicals Dashboard. The CATMoS collaboration provides an illustration of how the concepts of the strategic roadmap can be implemented.

Clarifying questions and comments:

Dr. Mansouri responded to several questions posed by members of the committee. When asked about how OPERA pKa models deal with chemicals with more than one pKa by Dr. Bolger, Dr. Mansouri responded that it provides the most acidic and most basic pKa for a chemical. The training set for these models are available as supplemental data from both a publication[10] and the OPERA page on the NIEHS GitHub repository[11]. In response to a question from Dr. Coleman, Dr. Mansouri stated that OPERA accepts multiple kinds of chemical identifier inputs (SMILES strings, DSSTox identifiers, CASRNs, etc.), and each LD50 prediction has an accuracy index. Dr. Gehen posed a question related to situations where correlation between acute lethal toxicity and structure is lacking. Dr. Mansouri replied that adding more data or building local models geared toward specific regions of chemical space generally resolves the issue. Dr. Kleinstreuer added that one goal of NICEATM's

[10] Mansouri et al. 2019. Journal of Cheminformatics. https://jcheminf.biomedcentral.com/articles/10.1186/s13321-019-0384-1
[11] https://github.com/NIEHS/OPERA

interactions with agencies is to understand how successful these models are in different chemical spaces and where additional data may be needed.

New Approach Methodologies for Exposure from EPA's ExpoCast™ Project

Dr. Kristin Isaacs, EPA, is co-lead of the ExpoCast project, which focuses on the exposure and toxicokinetics aspects of risk assessment. Evaluating chemicals for risk to humans or the environment requires information on hazard and exposure potential. Exposure potential quantifies the degree of contact between a chemical and a receptor. However, toxicokinetic information is required to predict the hazard based on the measured exposure (that is, what real world exposure is required to produce an internal concentration consistent with a potential hazard). One approach to dealing with the inherent uncertainty in estimating hazard through high-throughput outputs is to compare the hazard range to the range of expected exposure. Chemicals for which these are far apart are of less concern than those that are closer together or overlap.

Forecasting exposure is a challenging systems problem, as chemicals have diverse sources and pathways through the environment. ExpoCast characterizes four key pathways: consumer, occupational, ambient, and ecological. Because contact between chemical and receptor is not observable, measuring exposure requires indirect approaches such as building exposure models or monitoring exposure through sampling and inference. As collecting traditional exposure data is resource-intensive, ExpoCast relies on in silico approaches, or NAMs, to fill this data gap.

Dr. Isaacs reviewed the classes of NAMs used to predict exposure as described in her recent paper[12]:

- Chemical descriptors (e.g., EPA's Chemical and Products Database or CPDat) that provide information on chemicals in an exposure context.
- Machine learning approaches that use the chemical descriptors to fill gaps in existing data.
- High-throughput models for different exposure pathways including consumer products, dietary exposure for packaging, and water concentrations.
- High-throughput measurements, both targeted and non-targeted, to fill gaps in monitoring data.
- High-throughput approaches for measuring and predicting chemical toxicokinetics.
- Classes of NAMs pieced together to provide tools for high-throughput chemical prioritization.

EPA evaluates exposure NAMs through their Systematic Empirical Evaluation of Models (SEEM) framework, which uses Bayesian methods to incorporate multiple pathway models into consensus predictions for 1000s of chemicals.

Dr. Isaacs showed data comparing predicted exposure concentrations with estimates of doses

[12] Wambaugh et al. 2019. Current Opinion on Toxicology. https://doi.org/10.1016/j.cotox.2019.07.001

needed to cause bioactivity for 50 chemicals monitored by the National Health and Nutrition Examination Survey. Of these, very few had data indicating that the exposure and bioactivity concentration ranges might overlap, which would indicate the need for additional assessment and testing.

Clarifying questions and comments:

Several members of SACATM posed clarifying questions related to the models used by ExpoCast and the impact of high production volume chemicals on exposures. Responding to a question from Dr. Zhu, Dr. Isaacs stated that the EPA uses QSAR models for the chemical-use classification and mechanistic models for the pathway models. Sensitivity analyses and meta-analyses may also be used depending on the context. In response a question from Dr. Coleman about the relationship between production volume and, for example, chemicals found in house dust, Dr. Isaacs responded that the relationship is addressed through the human high-throughput exposure model, using rules-of-thumb to build a regression model. Production volume is only one predictor; other predictors include consumer use and industrial use. She cited methylparaben as an example, which would have high exposure because it is ubiquitous in consumer products but is a low production volume chemical.

Public Comments

Written Public Comments

One written public comment was submitted for this section, on behalf of HSUS.

Oral Public Comments

Dr. Esther Haugabrooks, representing PCRM, observed that the OECD AOP project and CATMoS are good examples of how collaborations can work to advance alternatives to animal use and lead to improved toxicity predictions. Based on discussions held between PCRM and stakeholders, Dr. Haugabrooks shared that stakeholders are seeking guidance on what assessment tools can be developed for regulatory use and what the regulatory requirements are. There was a concern that failure to implement computational tools is due to both a lack of understanding of their practical applications and confidence in these approaches. Other gaps impacting implementation include a lack of experimental data, including human data. Guidance, particularly guidance from agencies on fit-for-purpose applications, and training are needed to address the issues, especially as computational tools can be intimidating to those who are unfamiliar with their use.

PCRM encouraged the U.S. regulatory agencies to engage with OECD to ensure harmonization of new approaches and to share resources and knowledge. The regulatory agencies were also encouraged to engage in activities to diversify chemical space and data streams. PCRM recommended that SACATM support and advance the concept of data sharing and consensus modeling among domestic and international stakeholders. They also advised the agencies to adopt CATMoS for regulatory purposes, which will encourage the regulated community to increase utilization of such tools.

At a minimum, a flexible and transparent assessment framework for how data generated from computational tools can be used for regulatory purposes would be useful. PCRM and similar

organizations are working to raise awareness of the available tools and provide training opportunities.

Comments from Designated SACATM Discussants

Discussants for "New Approach Methodologies: Computational Tools" were Drs. Zhu, Williams-Devane, and Bolger. Addressing the first question related to current and future uses of machine learning models and barriers to use, Dr. Zhu commented that one drawback is the tendency to overfit models when data is limited. He acknowledged that this may be addressed somewhat with the creation of consensus models, but it is unclear how best to develop the consensus models and determine how many models are sufficient within the consensus model. Reflecting on Dr. Gehen's question to Dr. Mansouri on the utility of structure-based approaches to address activity cliffs, Dr. Zhu suggested that a better approach would be to simply introduce new data. However, this will be difficult to do without using additional animals. Dr. Zhu did not consider this much of a problem for acute toxicity endpoints, as there is existing data, but it will be a problem for more complex toxicities, such as developmental endpoints. Dr. Bolger cautioned against losing sight of the potential advantages of simpler models and added that the effectiveness of the machine learning models is limited by the available training data.

Responding to the question of how to best combine machine learning and mechanistic models to inform and improve one another, Dr. Williams-Devane suggested that the chemical properties be combined through a meta-representation in the input model prior to moving to the mechanistic model. Dr. Bolger described Simulations Plus' process where they use machine learning models to estimate chemical properties, which are used as inputs to the mechanistic models to predict movement and degradation of drugs. The models can be refined by supplementing sensitive parameters with experimental data.

Drs. Zhu and Williams-Devane identified three groups who might use ICE: users with limited expertise in computational tools who are looking for an answer to a specific question; computational biologists who want to look directly at the data; and data scientists with limited toxicology background who are developing models. ICE may be problematic for each of the three groups: ICE may be too complicated for the first group and frustrating to the second group because it is difficult to download the data, especially for the machine learning tool. It is also unclear what the data format is, for example, when you want to apply new algorithms. For the final group, there is currently insufficient guidance to ensure that their developed models will be useful. Dr. Bolger commented that ICE's limited support for chemical structures as both inputs and outputs is a major limitation. He added that the IVIVE tool needs more documentation to explain outputs, especially the column headings and output units.

The three discussants identified several opportunities for developing reference data sets. Dr. Zhu considered compiling a human reference data set a valuable, though labor intensive, exercise, and was unsure who would do this. Other data sets he thought of value included refence sets for predicting toxicities from mixtures and nanomaterials. He would be interested in participating in a challenge if a high-quality data set were developed. Dr. Williams-Devane recommended engaging the biomedical research community for thoughts on how to incorporate non-traditional types of information. She added that developing a human reference data set provided an opportunity to

engage other communities for creative solutions. Many of the problems mentioned at this meeting were address in the development of electronic medical records. Dr. Bolger suggested that ICCVAM engage with contract research organizations to generate data, especially for non-drug chemicals. He also commended NICEATM on its curation of the CATMoS dataset and its excellent job of finding data.

Dr. Zhu thanked the organizers for dedicating a full session to computational approaches.

Additional SACATM Comments

Dr. Hamadeh noted that today's talks highlighted the data curation work that makes downstream analyses possible. He proposed two approaches to predict toxicity of mixtures: 1) study the effects of the individual mixture components, or 2) model real-world evidence such as clinical data as an opportunity to show how molecules work together in a human system. These data could be combined with biomarker data from oncology or other contexts. It might be worthwhile to explore collaborations that could produce these types of data. Dr. Coleman suggested the available genotoxicity data as a data-rich-area for collection.

Response to SACATM Comments

Dr. Kleinstreuer thanked all the discussants for their valuable suggestions, especially the feedback on how to improve ICE downloadability and documentation. NICEATM would like to take advantage of the expressed interest to increase engagement with SACATM members throughout the year. Perhaps Drs. Zhu and Williams-Devane might facilitate NICEATM interaction with their students to help improve ICE documentation and training resources. The ICE chemical characterization tool will soon incorporate chemical product information from CPDat so users can visualize chemical use and product category coverage.

Dr. Isaacs noted that all machine learning models, training data sets, and predictions described in her talk are either currently available via the Chemicals Dashboard or will be within the next couple of months. Look for the "Exposure" tab on the dashboard. EPA is developing web services to customize delivery of exposure data to users.

After thanking all the day's speakers and commending the progress represented by the presentations, Dr. Spencer adjourned the meeting for the day at 4:23 p.m.

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Dr. Spencer called the second day of the meeting to order at 9:02 a.m. Participants introduced themselves and Dr. Maull reviewed meeting logistics and read the conflict of interest statement.

VII. New Approach Methodologies: Translational Impact and Human Relevance of Microphysiological Systems

Introduction

Dr. Berridge began his comments with the observation that toxicology is evolving from an

observational to a predictive science with a need to improve efficiencies and gain a better understanding of human biology. He added that microphysiological systems (MPS) represent an application that is poised to meet those needs. While the past dependence on animal studies has several logical bases, they fail to represent preexisting human disease and disease-chemical interactions, an area in which MPS can help.

The evolution of the Tox21 program from a tool to investigate individual receptor activity to one that can interrogate more complex interactions required an advancement in computational tools to enabled construction of more complex models of biological activity. However, the higher the throughput system, the further removed the study gets from the biological complexity of in vivo systems. AOPs can both be applied to better understand pathobiology and create a link between physiology at the molecular level and clinical outcomes.

While animal extrapolations are imprecise, current approaches to predictive toxicology are even more imprecise. MPS present an opportunity to build a better bridge between mechanistic understanding and in vivo outcome. Dr. Berridge outlined a potential strategy for implementing MPS, emphasizing that value will come not only from validation but also from qualification. The lung-on-a-chip system developed by the Wyss Institute, one of the first MPS systems, was influential in developing interest in MPS because of its functional capabilities and representation of physiology. Another element in building confidence is in vivo qualification, which has spurred discussion of whether animal studies are needed to inform new approaches and build confidence. Dr. Berridge concluded by commenting on the "hype cycle." There is currently a lot of interest and enthusiasm in MPS. We need to capitalize on that to push this technology forward.

Clarifying questions and comments

In response to a question from Dr. Milchak about how to address the challenge of accurately representing the concentration of a molecule at the target in in vivo systems, Dr. Berridge noted that computational tools can be applied to understand dose-response relationships and build extrapolations to in vivo systems. While MPS still represent a simplification of biology, they improve upon high-throughput systems. MPS not only allow us to control interactions that inform the kinetics of those relationships, but also allow us to modulate MPS to represent different levels of reserve capacity, in effect modeling a patient under duress.

The NIH Microphysiological Systems Program

Dr. Tagle introduced the National Center for Advancing Translational Sciences (NCATS) and its role in addressing challenges in drug development. The MPS program began in 2012 as a partnership between NIH, the DoD Defense Advanced Research Projects Agency (DARPA), FDA, and drug companies, with the goal of creating in vitro models for 10 human systems that were physiologically relevant, genetically diverse, and pathologically meaningful. The initial two funding opportunities from NCATS, the NIH lead institute for MPS, addressed platform and cell resource development, and multi-organ integration, physiological validation, and development of a training set of compounds. Dr.

Tagle provided examples of how MPS can reproduce organ function and address experimental requirements such as functional readouts, as well as how individual systems can be combined for multi-organ integration. A review of the diversity of projects showed the breadth of collaborators, which include academics, other NIH institutes, start-ups, the space program, and the pharmaceutical industry via the IQ Consortium, made up of 23-member companies.

The Tissue Chip Validation Framework, composed of two Tissue Chip Testing Centers at the Massachusetts Institute of Technology (transitioning to Javelin Biotech) and Texas A&M Tissue Chip Testing Consortium, and the MPS Database at the University of Pittsburgh, is tasked with building confidence in MPS in a phased approach. Phases progress through physiological validation (evaluating function and structure), analytical validation (evaluating robustness, reproducibility, reliability, and relevance), and industrial validation (use by industry and regulatory agencies, with proprietary compounds, in a CRO-type environment). The MPS Database Center integrates MPS data with preclinical and clinical data, all of which will be made publicly available. The Database Center also develops analytical tools and computational models of disease and toxicity.

Phase two of the tissue chip program is focused on disease modeling and efficacy testing. Current diseases of interest include common diseases such as Parkinson's, influenza, COPD, and diabetes, as well as several rare diseases. In partnership with the National Aeronautics and Space Administration (NASA) and the International Space Station National Laboratory, NCATS recently initiated its Chips in Space program to investigate physiological changes that take place under microgravity. These changes are of interest as they mimic aging but are reversible upon return to Earth. Future projects plan to study candidate therapeutics that slow these physiological changes. The technical aspects of developing MPS for use in space also support industrial validation. Dr. Tagle reviewed details of ongoing Chips in Space projects and their platforms.

Dr. Low continued the presentation by describing NCATS process for building confidence in MPS. There are many participants in this effort and many ongoing collaborations. Lessons learned through the evolving program include:

- Clearly identifying gaps and opportunities, creation of partnership, and involve end-users from the start
- Providing researchers with a supportive environment that includes resources, information, opportunities for formal and informal interaction, and guidance
- Building in procedures to minimize the impact of expected setbacks and failures by awarding milestone-driven grants and soliciting feedback to help guide progress, improve the process, and identify new opportunities

Future initiatives aim to take advantage of stem cell and genetic editing technologies, and focus on combining systems, rare diseases, and developmental/pediatric responses to drugs. Current funding opportunities explore the potential of using tissue chips in clinical trials, blood-brain barrier,

nociception, immune response, and Alzheimer's disease and dementia. Dr. Low closed by reviewing ongoing MPS development partnerships with other U.S. federal agencies and countries.

Clarifying questions and comments

Dr. Coleman asked the presenters to clarify the terms "precision medicine" and "personalized medicine." Dr. Tagle replied that both terms refer to using MPS to assess the efficacy of therapeutics in individuals without the drawbacks and hazards of clinical trials. The hope is that using MPS will reduce costs and identify who the best responders are.

In response to a question from Dr. Bolger about the logistics and limitations of conducting a 10-day experiment on a four-week spaceflight, Dr. Low commented that researchers need plan things out carefully, taking into consideration safety, fluid limits, and NASA's schedule and recognize the limitations within which their experiments must be conducted. Researcher develop and run multiple backup plans. Some cellular changes can be observed immediately and within the limits of a 10-day experiment in space.

Dr. Bolger asked if MPS models have enough accuracy to model the effect of a single nucleotide polymorphism on an organ system for situations where a drug target is highly genetically variable. Dr. Low cited an example where these sorts of effects have been modeled in the kidney.

Responding to a question asked by Dr. Williams-Devane regarding identified near-term gaps in computational needs, Dr. Tagle indicated that the Database Center's current areas of focus are in vitro to in vivo correlations and creating iterative computational models that learn and improve as more preclinical and clinical data are added. Machine learning and systems pharmacology approaches are being used to accomplish this. A number of these studies have been published.

In Vitro Microphysiological Systems at FDA

In introducing FDA's MPS program, Dr. Fitzpatrick provided related background on the FDA Toxicology Working Group, which is made up of senior toxicologists from FDA's 6 program offices and National Center for Toxicological Research (NCTR) and the Office of the Commissioner. This group was tasked to develop a roadmap to integrate emerging predictive toxicological methods and new technology into regulatory risk assessment. Their product, FDA's Predictive Toxicology Roadmap, was published in 2017. The goals of the Roadmap were to identify critical priority activities for new or enhanced FDA engagement to transform the development, qualification, and integration of new toxicology methodologies and technologies into regulatory application. The Roadmap will enable FDA to fulfill its current regulatory mission while preparing for the challenges of tomorrow. Building confidence in new tools is an important aspect of innovation that requires regulatory engagement in their development from inception.

In response to stakeholder requests voiced at a 2018 public hearing to solicit comments on the roadmap, FDA has:

- Published the first of their annual progress reports[13].
- Identified a single entrance point to present new methods to FDA (alternatives@fda.hhs.gov).
- Initiated development of a clear implementation plan through an intra-agency In Vitro Safety Working Group (IVSWG)

IVSWG is responsible for monitoring progress, disseminating information, and fostering communication and science applications for in vitro model partnerships. As such, IVSWG's first efforts will focus on developing and evaluating MPS for regulatory use.

Dr. Fitzpatrick reviewed the history of FDA's involvement with MPS, highlighting FDA's in-house work and collaborations with external partners, especially with Emulate and DARPA. These collaborations have developed a liver model; other organs and applications of interest are a gut model and a neuromuscular junction model for botulinum toxin testing. Ongoing MPS projects within FDA offices and centers include:

- Evaluation of a liver-on-a-chip and a heart-liver system (Center for Drug Evaluation and Research).
- Practical microscale biomimetic model development to test regenerative medicine products and in vitro models of complex systems such as the tumor microenvironment and blood vessel generation (Center for Biologics Evaluation and Research)
- Gut model development with the short-term goal of assessing the effect of antimicrobial drug residues on the human microbiome and longer-term goal to qualify these models for the evaluation of antimicrobials for food animal use (Center for Veterinary Medicine).
- Placenta and at liver model development (the latter to support rat-to-human extrapolation) (NCTR).
- Exploration of several organ models to study radiation damage to replace nonhuman primate studies (FDA's Medical Countermeasures Initiative).

The goals of the IVSWG MPS program are to define terminology; identify research and regulatory gaps; foster partnerships to advance technology; establish draft performance criteria; and develop a Request for Information for developers and end users. FDA is engaged in discussions with international counterparts to explore the formation of a group of global regulators to advance these systems. Dr. Fitzpatrick noted that while formal programs for qualification of tools exist within the FDA centers for devices and drugs, FDA encourages researchers and regulators engage in informal discussions with stakeholders about the feasibility of qualifying an alternative tool for a specific purpose. FDA accepts nontraditional methods for several applications and is providing training to regulators to help them become familiar with new methods and encourage acceptance. A webinar series convened by the Office of the Chief Scientist allows developers to present new methods to FDA scientists. To be selected to participate in the webinar series, developers must submit descriptions of the method, the proposed context of use, and what regulatory issue or gap it could

[13] Available at https://www.fda.gov/media/128045/download

address; data and publications are also requested.

Clarifying questions and comments:

In response to a question from Dr. Clippinger, Dr. Fitzpatrick indicated that a time frame for releasing performance criteria had not yet been established.

Dr. Coleman asked about the FDA's current thinking on tattoos. Dr. Fitzpatrick noted that while pigments and artists are not regulated by FDA, they are of concern, especially for women of childbearing age. FDA cannot act in non-regulated areas such as tattoos and dietary supplements unless there is some evidence of harm, which puts the burden of proof and the need to do the research on FDA. Emulate is working with a manufacturer to model skin penetration and systemic uptake of inks.

Beyond 3D Models: Building Confidence in Microphysiological Models

Dr. Szczepan Baran of Novartis praised SACATM for bringing people from different areas together into the broad collaboration that is needed to advance MPS. MPS are advancing rapidly relative to other biological technologies. The current challenge is to define the potential of the technology and determine the best applications. Context of use is a key area, as well as facilitating appropriate partnerships and assessing performance.

With the number of different systems under development, evaluation becomes difficult as different models have different properties. The North American 3Rs Collaborative has established a working group on complex in vitro models. They are compiling information about the different systems to enable easy system comparisons by users. The group is also envisioned as a forum through which stakeholders can communicate.

Dr. Baran emphasized the need to address technical issues, such as the impact of different component materials on system performance. The NCATS MPS Database and Tissue Chip Testing Centers are playing important roles in analyzing and addressing technical concerns. The concerns brought up in the previous session about data usability by the variety of users applies here also.

Dr. Baran described his role at Novartis as working with scientists to identify and apply new technologies. Considerations included in this process are system duration (set-up time, viability, activity), system characteristics (cell composition, function, maintenance level, etc.), abilities (frequency and type of sampling, imaging options), testing parameters (cell and media sourcing), endpoints and their performance, and restrictions. Addressing these considerations can help determine the suitability of a system for an application. He identified potential contexts of use for MPS at each stage of the drug development timeline. Safety testing at the preclinical stage is an obvious application but this may not be readily adopted because of the regulatory implications of results. Applying MPS in earlier research stages may be easier to achieve. Other potential applications for MPS include modeling orphan diseases, precision medicine, and nanomedicine.

Dr. Baran identified issues that will need to be addressed to enable full utilization of MPS. Scalability

and complexity of the systems will affect their utility and applicability. Standards need to be established for data from MPS. The potential utility of animal-based MPS should not be overlooked, as these might be more readily adopted for preclinical applications. Balancing complexity and practicality are dependent on the application. Other key aspects to factor in include reproducibility, reliability, robustness, and relevance. Transferability of these systems can be limited, but, as the Chips in Space program has demonstrated, a high degree of robustness can be achieved. Adoption of MPS is dependent on demonstrating that these new technologies have enough value to justify the developmental time and resource costs. Collaboration is key, especially parallel engagement with international regulatory bodies.

The IQ Consortium MPS affiliate is focused on addressing precompetitive challenges. They anticipate publication of several papers over the next six months, each covering a different organ and describing industry context of use vision and features needed to address that context of use. IQ also has groups focused on regulatory and non-regulatory engagement. IQ is aware of the need to collaborate with stakeholders outside of the pharmaceutical industry, and to that end is pursuing interactions with the NA3Rs Collaborative. MPS technologies are also being actively pursued within the environmental community and Dr. Baran recommended increased interaction with them in the future.

Clarifying questions and comments:

In response to a question from Dr. Casey about the relationship between the IQ MPS affiliate and the tissue chip validation centers, Dr. Baran indicated that the IQ MPS affiliate is currently focused on developing publications that describe the use of MPS within the industry, rather than doing actual projects with the technology. The two groups also address different topics.

Dr. Berridge reiterated the session's goals of providing a background for MPS and summarizing ongoing activities and challenges. This technology is maturing but now needs to be aligned to specific contexts of use. Citing the pharmaceutical industry, he noted how progress in safety assessment has often been achieved in response to specific needs. He asked SACATM to consider and suggest specific applications and collaborations that could drive MPS development forward.

Public Comments

Two written public comments were submitted for this section, on behalf of HSUS and PCRM.

Oral Public Comments

Dr. Elizabeth Baker, representing PCRM[14], asked SACATM to encourage agencies to focus on how to integrate these technologies into regulatory decision-making. She cited the FDA new tools qualification programs as examples of how an agency can facilitate both collaborations to qualify new technologies and communication about their use.

[14] Dr. Baker provided her comments by phone.

Comments from Designated SACATM Discussants

Drs. Charest, Hamadeh, and Milchak were the assigned reviewers for the session titled New Approach Methodologies: Translational Impact and Human Relevance of Microphysiological Systems. The three reviewers agreed that chemical screening, whether for drug candidates or for chemical toxicity, was a likely opportunity. As stated by Dr. Hamadeh, the need to address problems that currently have no satisfactory solution will drive how MPS applications are developed. Dr. Charest and Milchak added that MPS could fill testing needs in applications when animal models for humans are either not relevant or do not exist, when data between humans and animals are in conflict, or when the utility of computational approaches might be limited. Dr. Charest provided a list of potential uses of MPS including assessing tissue- and organ-level effects; examining high-risk situations or sensitive populations; conducting pharmacokinetic studies that link multiple tissues together; and identifying training sets for machine learning or refining the training sets by generating data to fill gaps. Dr. Milchak added that running MPS applications in parallel with animal tests to provide supporting data would be most useful.

Utilizing MPS in efficacy screening will reduce animal use and possibly improve decision-making, in Dr. Hamadeh's opinion. Dr. Charest added that an animal-on-a-chip as a model could be more easily validated than a human model and thus replace animal use in the short term. Dr. Hamadeh suggested that confidence will improve as more data emerge from consistent models. This should ultimately impact animal use.

While Dr. Charest stated that developers need to clearly understand how the technology will be implemented when the validation criterial are met, Dr. Hamadeh commented that a clear threshold needs to be defined so that users know what success looks like, and the regulators need to agree on vision and context. Dr. Milchak supported the idea of focusing MPS' development around specific contexts of use, especially from a regulatory perspective. Dr. Charest commented that goals and outcomes should be publicly available and reporting both successes and failures should not only be allowed but incentivized. Dr. Hamadeh added that identified limitations should be clearly stated.

The reviewers provided an extensive list of overarching needs for the implementation of MPS. In Dr. Milchak's opinion, no single system will replace animal use; however, MPS holds the most promise to fill that gap, especially when used in conjunction with other technologies. Dr. Hamadeh cautioned against advertising MPS as standalone technologies; they should be used in conjunction with other approaches, especially computational approaches. Key to MPS implementation are defining the context of use and validating the systems. Dr. Charest suggested that the context of use be focused while addressing diverse applications at the same time. Dr. Hamadeh sought clarity on what "validation" means – does it mean that the MPS can replicate physiology, for example, or identify bad actors? To identify bad actors, MPS will need to fully represent in vivo physiology. Additional validation, possibly involving animal studies, will be needed to demonstrate that MPS can predict toxicity. Other needs include demonstrated scalability and consistency across assay formats. The availability of curated and usable validation or training data would be very helpful.

Dr. Milchak cautioned against reinforcing an impression that the science is further along than it really is. The value of these models is not clear because the technology is still nascent and cost-prohibitive

for many, added Dr. Hamadeh. He also stated that it is hard to envision what MPS' impact will be given the dearth of data on confidence. As MPS are highly specific and highly mechanistic, Dr. Milchak was concerned that organ system interactions or other hazards might be missed due to highly specific conditions of systems. Dr. Charest noted that sourcing cells and tissues might be tricky, especially for healthy tissues, though stem cell technology may address this issue. Once specific technology gaps have been identified, funding agencies can focus resources to fill the gaps. Dr. Hamadeh added that funding in diverse spaces will support broader proficiency, better understanding of what the models can do, and greater innovation.

The reviewers agreed that a plan for building confidence or a roadmap that would facilitate the development an adoption of MPS would be useful.

Additional SACATM Comments

Dr. Clippinger reiterated support for applying MPS to identify effects in humans that aren't seen in animals. Our current approach to validation of new methods is undermined by trying to correlate the performance of a new method to an existing animal method without considering human data. In that case, it's hard to interpret lack of concordance between the new method and the animal method.

In response to a question posed by Dr. De Abrew, Dr. Berridge commented that it would be difficult to start with an MPS as an initial screen for a chemical with no other data because, as has been stated, the systems may be too specialized. Currently, the best opportunity for MPS use is in situations where some data exists.

Dr. Spencer commented on the need to develop technical expertise in using MPS as we progress towards applying them to safety and regulatory testing. Using them in basic research applications might help with this. She suggested developing a challenge for universities that could help identify practical applications.

VIII. Adjournment

Dr. Casey thanked everyone for their attendance and participation. The evolution of this committee over the last five years has been remarkable and has in large part been due to the efforts of those serving on the committee. NICEATM and ICCVAM have listened to SACATM's contributions and have acted on their recommendations. Dr. Berridge echoed those sentiments and especially appreciated the feedback on MPS.

Dr. Spencer adjourned the meeting at 12:50 p.m.

Signed Jan. 28, 2020

Pamela J. Spencer, PhD, DABT

SACATM Chair

Date: