Summary Minutes
September 24, 2013 SACATM
NIEHS, Research Triangle Park, NC

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I. Location of Background Materials/Presentations and Frequently Used

Abbreviations

Background materials and presentations for the 2013 Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) meeting are available on the SACATM meeting website (*http://ntp.niehs.nih.gov/go/8202*).

3Rs	Replacement, reduction, and refinement (causing less pain and distress) in the use of animals for toxicological testing
AOP	Adverse Outcome Pathway
aP	acellular pertussis
ARDF	Alternatives Research & Development Foundation
ASPCA	American Society for the Prevention of Cruelty to Animals
CHO	Chinese hamster ovary
CPSC	Consumer Product Safety Commission
CVB	Center for Veterinary Biologics
DABT	Diplomate of the American Board of Toxicology
DARPA	Defense Advanced Research Projects Agency
DOI	Department of the Interior
ECVAM	European Center for the Validation of Alternative Methods
EASA	electrophilic allergen screening assay
EDSP	Endocrine Disruptor Screening Program
ELISA	enzyme-linked immunosorbent assay
EPA	U.S. Environmental Protection Agency
EU	European Union
EURL	The European Union Reference Laboratory
FDA	U.S. Food and Drug Administration
HIST	histamine sensitization test
HTS	high throughput screening
HSUS	The Humane Society of the United States
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ICATM	International Cooperation on Alternative Test Methods
ICH	International Conference on Harmonisation
ILS	Integrated Laboratory Systems, Inc.
ITS	integrated testing strategy
JaCVAM	Japanese Center for the Validation of Alternative Methods
KoCVAM	Korean Center for the Validation of Alternative Methods
MOU	memorandum of understanding
NAS	National Academy of Sciences
NICEATM	NTP Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences

NIOSH	National Institute for Occupational Safety and Health
NIH	National Institutes of Health
NLM	National Library of Medicine
NTP	National Toxicology Program
OECD	Organisation for Economic Cooperation and Development
OSHA	Occupational Safety and Health Administration
PCRM	Physicians Committee for Responsible Medicine
PETA	People for the Ethical Treatment of Animals
qHTS	quantitative high throughput screening
RFP	request for proposal
SACATM	Scientific Advisory Committee on Alternative Toxicological Methods
SAR	structure-activity relationship
SBIR	Small Business Innovative Research
SSS	Social and Scientific Systems, Inc.
USDA	U.S. Department of Agriculture

II. Attendance

SACATM met on September 24, 2013, at the National Institute of Environmental Health Sciences (NIEHS) in Research Triangle Park, North Carolina. The following individuals attended the meeting:

SACATM

Lauren Black, PhD, Charles River Laboratories
Tracie Bunton, DVM, PhD, Eicarte, LLC
Joy Cavagnaro, PhD, DABT, ATS, Access BIO, L.C.
Joan Chapdelaine, PhD, Calvert Laboratories
Michael Kastello, DVM, PhD, Sanofi
Safdar Khan, DVM, MS, PhD, DABT, ASPCA
Steven Niemi, DVM, DACLAM, Harvard (SACATM chair)
Ricardo Ochoa, DVM, PhD, ACVP, Pre-Clinical Safety, Inc.
Michael Olson, PhD, ATS, GlaxoSmithKline
Linda Toth, DVM, PhD, DACLAM, Southern Illinois University School of Medicine
Daniel Wilson, PhD, DABT, The Dow Chemical Company

ICCVAM Principal Representatives

Surender Ahir, PhD, OSHA (by videoconference)
Carol Clarke, DVM, DACLAM, USDA (by videoconference)
Pertti Hakkinen, PhD, NLM
Abigail Jacobs, PhD, FDA, ICCVAM Co-Chair
Christine Kelley, PhD, NIH
Anna Lowit, PhD, EPA, ICCVAM Co-Chair
Joanna Matheson, PhD, CPSC

Paul Nicolaysen, VMD, NIOSH
Barnett Rattner, PhD, DOI (by videoconference)
Raymond Tice, PhD, NIEHS/NTP

Other ICCVAM Representatives
Jeanne Goshorn, MS, NLM
Richard McFarland, MD, PhD, FDA
Stephanie Padilla, PhD, EPA
Jeffrey Patton, FDA (by videoconference)

Invited Speakers
Geetha Srinivas, DVM, PhD, USDA (by telephone)
Nicole Kleinstreuer, PhD, ILS

NIH/NIEHS Staff
Linda Birnbaum, PhD, DABT, ATS, NIEHS/NTP Director
John Bucher, PhD, NTP Associate Director
Warren Casey, PhD, DABT, Acting Director, NICEATM, Acting Administrative Director, ICCVAM
Kelly Chandler
Robbin Guy
Robin Mackar
Elizabeth Maull, PhD
Mark Miller, PhD
Sheila Newton, PhD
Mary Wolfe, PhD, NTP Deputy Director for Policy
Lori White, PhD, PMP, SACATM Designated Federal Officer
Yun Xie, PhD

Bridport Services, LLC
Ernie Hood, MA

ILS (NICEATM support contractor) Staff
David Allen, PhD
Steven Morefield, MD
Lori Rinckel, PhD
Catherine Sprankle
Judy Strickland, PhD, DABT

Public
Aryenish Birdie, PCRM
Jeffery Brown, PETA

Amy Clippinger, PETA
Yoshihito Deguchi
Jack Fowle, PhD, Science to Inform
Brian Jones, Stiefel/GlaxoSmithKline
Sue Leary, ARDF (by telephone)
Richard Morris, SSS
Jason Pirone, SSS
Marjo Smith, SSS
Catherine Willett, PhD, HSUS

III. Welcome and Opening Remarks

SACATM Chair Dr. Steven Niemi called the meeting to order at 9:00 AM. All in attendance introduced themselves. Dr. Niemi welcomed the new SACATM members, Drs. Lauren Black, Michael Kastello, and Safdar Khan. He noted that SACATM members Drs. Marilyn Wind and Mark Evans were unable to attend the meeting.

NIEHS/NTP Director Dr. Linda Birnbaum welcomed everyone to the meeting, including those attending via videoconference. She described the changes at ICCVAM and NICEATM since the last SACATM meeting, including the retirement of Dr. William Stokes, the past Director of NICEATM and Executive Director of ICCVAM. She noted that Dr. William Casey, previously Deputy Director of NICEATM, is now Acting Director of NICEATM and Acting Administrative Director of ICCVAM. She introduced one of the meeting's main topics, the new vision and directions for ICCVAM, which includes a new philosophy of member agencies driving the ICCVAM agenda for ICCVAM's priorities. Also, she noted that the role of NICEATM would be expanded, including interfacing it more closely with the Tox21 initiative and helping to integrate new data and methods into the regulatory framework. She recognized and thanked the SACATM members whose four-year terms were ending with this meeting and awarded certificates of appreciation to Drs. Michael Olson, Steven Niemi, and Linda Toth.

NTP Associate Director Dr. John Bucher added his welcome to the meeting attendees. He stated that over the past year the opportunity had arisen to re-examine ICCVAM's procedures and resources, as well as its fundamental goals, and at this meeting a new vision for ICCVAM would be presented to SACATM. He thanked the Federal agency representatives to ICCVAM for their hard work and dedication, and Dr. Niemi for his steady hand in chairing the SACATM proceedings.

ICCVAM Co-Chair Dr. Abigail Jacobs, FDA, noted that this would be the first SACATM meeting since initiating revision of the roles, responsibilities, and operating procedures for ICCVAM and NICEATM, and that the changes were still a work in progress.

Designated Federal Officer Dr. Lori White read the conflict of interest statement for SACATM.

IV. New ICCVAM Vision and Procedures

A. Introduction

Acting NICEATM Director and ICCVAM Acting Administrative Director Dr. Casey briefed SACATM on the new ICCVAM vision and procedures. He introduced the key themes of efficiency, collaboration, and innovative thinking related to the ICCVAM changes in vision and focus.

Dr. Casey said one major element of the administrative changes is to define his roles in ICCVAM and NICEATM. Dr. Casey will serve as acting administrative director of ICCVAM (and not a voting member of ICCVAM) and Dr. Raymond Tice will be the NIEHS principal representative to ICCVAM. Other changes include more frequent, shorter ICCVAM meetings and the management of peer reviews through the NTP Office of Liaison, Policy and Review. Agency needs will now drive ICCVAM activities and they will be brought to ICCVAM for consensus approval.

Dr. Casey reviewed the ICCVAM Authorization Act of 2000, including its stated purposes, which are (1) increase the efficiency and effectiveness of Federal agency test method review; (2) eliminate unnecessary duplicative efforts and share experiences between Federal regulatory agencies; (3) optimize utilization of scientific expertise outside the Federal government; (4) ensure that new and revised test methods are validated to meet the needs of Federal agencies; and (5) reduce, refine, or replace the use of animals in testing, where feasible. ICCVAM seeks to re-emphasize these purposes and to keep its original intent as a committee designed to review test methods, not to run validation studies. He described the new role for NICEATM, which has been expanded to provide scientific and operational support to ICCVAM, the NTP, and Tox21, which will allow NICEATM a new degree of operational flexibility to facilitate interagency and international collaboration. He noted the need for ICCVAM to coordinate or share information among Tox21, the National Center for Computational Toxicology, and the EPA Office of Research and Development, as well as non-ICCVAM programs such as the NIH-Defense Advanced Research Projects Agency (DARPA) *Organ-on-a-Chip* initiative. Dr. Casey noted that the emphasis in ICCVAM activities going forward would be on cooperation and collaboration.

He described the ongoing importance of close coordination with international partners such as the International Cooperation on Alternative Test Methods (ICATM) and the Organisation for Economic Cooperation and Development (OECD).

B. A New Vision and Direction for ICCVAM

Interim ICCVAM Co-Chair Dr. Anna Lowit, EPA, described the draft document titled *A New Vision and Direction for ICCVAM.* This document outlines the initial steps toward a new strategic direction for ICCVAM and NICEATM and was provided to SACATM members and the public for feedback. The draft covers three areas: (1) ICCVAM priority setting and science focus areas for immediate ICCVAM resource investment, (2) plans to improve communication with stakeholders and the public, and (3) exploration of new paradigms for the validation and utilization of alternative toxicological methods.

The change in approach in ICCVAM's priority setting will streamline the number of active projects, focusing on projects with a reasonable likelihood of success within a reasonable timeframe (1-5 years), while maintaining flexibility to reorient efforts as needed. Initially, three short-term projects are identified: (1) biologics (*Leptospira* vaccine potency), (2) acute oral and dermal toxicity testing, and (3) skin sensitization. ICCVAM is also developing revised procedures for the submission or nomination of new assays or projects. To move forward, a proposed project must be sponsored by at least one Federal agency. There will be more emphasis placed on working with test method developers and reviewing validation study data, as opposed to NICEATM conducting validation studies on behalf of test method developers.

Dr. Lowit described the three identified project areas in more detail, and then discussed plans to improve ICCVAM's communication efforts, including improving the ICCVAM website and achieving broader engagement with the scientific community and stakeholders through a variety of methods. Also, there will be an effort to increase agency awareness of international 3Rs initiatives.

As part of its efforts to explore new paradigms, ICCVAM recognizes the need for an evolving definition of "validation" that is responsive to new technologies and ongoing paradigm shifts in toxicity testing. ICCVAM plans to better align with the vision espoused in the 2007 NAS report, *Toxicity Testing in the 21st Century* and to promote development of test batteries as opposed to one-for-one test replacements.

C. Public Comments

Dr. Catherine Willett, representing the Humane Society of the United States (HSUS), presented comments on *A New Vision and Direction for ICCVAM*. She said HSUS is excited about the new roles of NICEATM and ICCVAM.

In terms of prioritization, the HSUS suggests ICCVAM maintain close partnership with the agencies for which the method is applicable throughout the validation process, including creating a validation oversight committee composed of members from each applicable agency. It might also be helpful, in the context of limited resources, for

ICCVAM to categorize projects as those in which ICCVAM will take the lead, those in which ICCVAM will serve as an active partner, and collaborative projects in which ICCVAM will function in a support role.

Under improving communications, Dr. Willett suggested adding content on the ICCVAM website to bring greater visibility to agency activities regarding the 3Rs. Additionally, there should be both more ICCVAM-led workshops and webinars, along with more regular participation by ICCVAM members in workshops and other scientific meetings held by others.

Regarding new paradigms for regulatory acceptance and utilization of alternative methods, Dr. Willett said ICCVAM should seek improved international coordination, move toward integrated strategies such as OECD's Adverse Outcome Pathway (AOP) approach, and consider statistical approaches to validation, such as Bayesian network approaches. Also, different evaluation processes for different applications should be considered.

Ms. Sue Leary, President of the Alternatives Research & Development Foundation (ARDF), commented by telephone. She said ARDF is very pleased to see ICCVAM's focus back to its original purposes, since they still represent a valuable framework and objectives. ARDF considered *A New Vision and Direction for ICCVAM* an encouraging development and particularly liked the expansion of the concept of validation beyond one-to-one replacement. They approve of the emphasis on productivity and transparency, and see the wisdom in the use of lead agency facilitating the progress of alternative methods. ARDF endorses the expansion of the ICCVAM website and the addition of the national coordinator to ICCVAM. All of these changes, she said, will strengthen ICCVAM's impact as toxicology moves into a new era.

Dr. Aryenish Birdie, representing Physicians Committee for Responsible Medicine (PCRM), commented on the new vision. She said in the past sometimes individual regulators within ICCVAM Federal agencies would reject data from non-animal test methods, although the agency's stated policy was to accept those methods. She said it is important that agency scientists understand the methods that ICCVAM and the European Center for the Evaluation of Alternative Methods (ECVAM) endorse and how those data can be used in order to ensure that the data companies submit using those alternatives are accepted. Otherwise implementation of non-animal test methods will be unsuccessful. She asked that ICCVAM release its raw data on oral-dermal LD_{50} evaluations to be transparent.

D. SACATM Discussion

Dr. Olson, first discussant, congratulated Dr. Birnbaum for making important strategic changes to the ICCVAM program. He said he would limit his comments to the focus and priorities of ICCVAM and NICEATM in addressing the discussion questions.

He noted that the timeline on changes in ICCVAM activities is relatively recent, starting in February 2013 with Dr. Birnbaum's editorial in *Environmental Health Perspective* titled *15 Years Out: Reinventing ICCVAM*. He said a carefully constructed, written, tactical plan to achieve the new ICCVAM strategy is needed. He found it encouraging that ICCVAM agency representatives have been meeting and discussing the new strategy. The tactical plan should define the new working relationship among ICCVAM member agencies. It should also address how to communicate and promote the new methods broadly to each ICCVAM member agency and beyond just the ICCVAM representatives. He felt the plan should include a strategy to enhance engagement with stakeholder groups outside of ICCVAM and define the future role for SACATM. Consideration should also be given to revising and republishing the deferred *2013-2017 ICCVAM Strategic Plan* in light of the new paradigms.

Dr. Olson said it would be critical for ICCVAM to emphasize its role as a member of the international 3Rs community by integrating its activities with ECVAM, Japanese Center for the Validation of Alternative Methods (JaCVAM), Korean Center for the Validation of Alternative Methods (KoCVAM) and the other international agencies under the ICATM partnership. That effort should include leveraging scientific resources and avoiding duplication.

He noted that the intent for improved communication by ICCVAM is exceptionally important. He favored revising the website and using other means of communication for outreach, not only to the ICCVAM community itself, but also other stakeholders with an interest in alternative testing methods.

With respect to the new ICCVAM operating model, he said this would be an appropriate time to step back and canvass the ICCVAM regulatory agencies about how animals are actually used. The focus should be on the entire breadth of product safety assessment.

Regarding the decision to require a Federal agency to sponsor a nomination or submission, Dr. Olson stated that it would be vital to construct and run a transparent process so that people in the partnering agencies understand how the ideas for projects that they put forward are prioritized. He recommended that the new paradigm include information about how to put forward an idea and how that idea will be evaluated.

Dr. Daniel Wilson, second discussant, noted his support for the new direction of ICCVAM and NICEATM toward a Tox21 approach that uses much broader mechanistic

and high throughput *in vitro* models as well as cheminformatics and statistical approaches. He noted that validation of the approaches would need to be addressed, including the appropriateness of any predictive mathematical models. He said a better understanding of coordinating the results of *in vitro* assays and chemical structure-activity models is needed and recommended ICCVAM draft a comprehensive plan for such efforts. He expressed support for ICCVAM's strategic decision to require a Federal agency to sponsor nominations. He approved of the three areas identified by ICCVAM as priorities going forward, but suggested that traditional areas such as skin and eye irritation and corrosion should still have a role. He urged that the data for any endpoint identified as a high priority be readily accessible in a curated, annotated, publicly available database, although he acknowledged that that would be no small task.

Dr. Wilson noted that *Leptospira* vaccine potency testing is rapidly trending toward success in the use of an enzyme-linked immunosorbent assay (ELISA) test. Regarding skin sensitization, he said scientific advances have largely enabled the success of high-throughput *in vitro* and cheminformatics approaches. He considered acute toxicity testing as the least developed and most challenging of the three priority areas.

Dr. Joy Cavagnaro, third discussant, quoted from the section of the ICCVAM Authorization Act focusing on the provision mandating that ICCVAM facilitate validation criteria and the acceptance of test methods by Federal agencies and other stakeholders. She said the new process for ICCVAM does not change those requirements. She felt the change in focus and priorities for ICCVAM, particularly the emphasis on regulatory agencies setting the agenda, is necessary and should improve implementation and acceptance of validated alternative methods. The new approach to promoting the 3Rs must be driven by regulatory agency needs. With success in the use of alternative methods inextricably linked to regulatory acceptance, there must be continuous efforts to recalibrate with regulatory expectations to ensure a reasonable timeline for acceptances. She added that having a good sense of each agency's needs would be important in priority setting. ICCVAM members responsible for regulated products should provide input during the early phases of nomination and throughout the validation process, perhaps through designation of a sponsor or "champion." Issuance of requests for proposals (RFPs) by agencies via ICCVAM, along with guidance for industry, would help to facilitate the process. She noted the importance of understanding the relationship between ICCVAM and global acceptance by OECD, the International Conference on Harmonisation (ICH), and ICATM. Additionally, the ICCVAM website should include a comprehensive summary of international efforts to implement the 3Rs. She felt it would be necessary to capture test utilization data as a metric of success, with industry groups establishing tracking systems to document use of alternative methods. She supported reconsideration of the concept of validation, particularly as a priority item over the next year.

Dr. Casey noted that the intent is not for ICCVAM to move away from validation, but rather, to reconsider how validation is achieved and approached. He said ICCVAM intends to make all data public, including oral and dermal, after quality control is completed on the data. Regarding a five-year plan, he said the technologies are changing so rapidly it becomes almost impossible to generate a useful five-year plan. Instead, ICCVAM will develop priorities, develop an initial strategy, solicit public comment, and then finalize a strategic plan for every project area.

Dr. Ricardo Ochoa noted that much of Dr. Birnbaum's *15 Years Out: Reinventing ICCVAM* editorial addresses the gap between alternative methods validation and regulatory agency acceptance and implementation. His concern with the new strategic direction for ICCVAM is that it shifts responsibility onto the agencies to come up with models to be validated and accepted, with no assurance that those organizations will change their priorities to facilitate that process. He asked what ICCVAM would do to expand the number of alternative models as well as their acceptance. He felt the 3Rs are generally a secondary concern among the agencies, with no commitment that they will work in that direction, and that the new ICCVAM strategy may not change the situation.

Dr. Cavagnaro noted that there have been some instances where animal methods were shown to be inappropriate and biologics had been quickly advanced into the clinic based on *in vitro* data. Thus, she said, industry needs to be more forthcoming when clear alternatives are developed and used, although they may not have been validated. Dr. Black echoed those comments, noting that irrelevant animal models that should be discarded. She felt that all animal models and *in vitro* models should be assessed to ensure that they are "fit to purpose." In that context, Dr. Ochoa noted a "particularly irksome" regulation – the FDA rule that there must be two carcinogenicity assays in order to regulate a compound. He noted that the EU had required only one, the rat, for many years. He suggested there be a study of such regulations by a group representing both industry and regulatory agencies, hopefully internationally, so that more of a consensus could be reached. Dr. Cavagnaro commented that these issues are currently being discussed at ICH, including the possibility that assessment of carcinogenic risk could achieved without conducting the two-year rodent carcinogenicity studies. Dr. Joan Chapdelaine emphasized the importance for industry working closely with the regulatory agencies to advance new alternatives.

Dr. Bucher noted that after the forthcoming presentation on Tox21, he would ask SACATM to think about how to address the issue of validation in the context of the new assays. Further, he would ask SACATM how they might want to be involved in revisiting the ICCVAM validation criteria developed in the 1990s. Dr. Black described validation in the context of regulatory and public policy setting and risk assessment; it is taking an assay considered representative of the problem to be solved, then resolving

any problems with the assay so that it works routinely and robustly. She felt ICCVAM should focus on identifying assays that are "fit for purpose" and promoting their development.

Dr. Niemi agreed with the call for a tactical plan and also pointed out a need for "tactical metrics," to help quantify the number of animals saved by using the alternative methods. Without those data, he said, credibility is being risked, because many people have been asking for those numbers and are waiting for them to emerge. He suggested using personnel similar the Service Corps of Retired Executives (SCORE) to help gather the raw data, conceiving the Enthusiastic Corps of Retired Toxicologists, or "ESCORT", who would volunteer to collect the raw data and analyze it. He added that it would be useful to consider using experts from outside the field to contribute new ideas.

V. Update on NICEATM Activities

A. Presentation

Dr. Casey updated SACATM on NICEATM, which provides administrative and scientific support to ICCVAM. He noted that like most government entities, NICEATM is currently operating under a "reduced financial situation." Under the vision for a re-invented ICCVAM, NICEATM, with 2 Federal employees and 12 contract staff, now provides support to the NTP and Tox21 as well. Retaining its core competency in validation study design and data analysis, NICEATM will add expertise in computational toxicology, cheminformatics, and data management through its contract. Redesign of the NICEATM and ICCVAM websites will also occur.

Dr. Casey said NICEATM will continue to organize workshops, but in the future would look for opportunities for collaboration and co-sponsorship. He described several upcoming workshops, including *Translational Alternative Models and Biomarkers Predictive of Drug or Chemical Cardiovascular Risk, Aquatic Models and 21st Century Toxicology*, and *Regulatory Applications of Adverse Outcome Pathways*.

NICEATM will continue to provide validation study support, although NICEATM cannot pay for validation studies. Also, NICEATM will continue to promote the Small Business Innovative Research (SBIR)/ Small Business Technology Transfer (STTR) grants to encourage innovation in validation studies technologies and other 3Rs-related research.

New focus areas for NICEATM include production of high quality *in vivo* reference data (e.g., a database of reference chemicals for assessing estrogenic activity), validation support for Tox21, and new computational approaches. Dr. Casey noted that a database of high quality *in vivo* data will be essential to fully use *in vitro* data.

To illustrate the new involvement with Tox21, he described the Tox21 10K library and provided details about how high throughput screening (HTS) assays are validated. Tox21 validation models are to be based upon manual-to-HTS, HTS-to-manual, and HTS-only protocols. New computational approaches will focus on developing methods for accurate extrapolation of *in vitro* data to *in vivo* activity.

Dr. Casey noted that NICEATM would also address other important focus areas such as metabolism, AOPs, integrated testing and decision strategies, and mixtures.

B. SACATM Discussion

Dr. Cavagnaro asked a clarification question to Dr. Casey regarding the term "chemical" when discussing Tox21. Dr. Casey replied that "chemical" is broadly defined and includes chemicals, drugs, and pesticides. Dr. Casey added that NICEATM had been asked to assist with the EPA's Endocrine Disruptor Screening Program (EDSP) 21 program that will incorporate new methods to assess endocrine activity.

Dr. Ochoa, first discussant, said NICEATM's scientific activities are consistent with the new operating paradigm outlined in Dr. Birnbaum's editorial *15 Years Out: Reinventing ICCVAM* and *A New Vision and Direction for ICCVAM*. He reiterated his concern about the level of participation by the regulating agencies. He asked how NICEATM would prioritize its activities. He favored the approaches described by Dr. Casey, but wondered whether they would actually result in a decrease in the number of animal tests, and in what species. Understanding the numbers of animals used in testing currently would inform prioritization of activities going forward in terms of ICCVAM's 3Rs mandate. He expressed his support for widespread dissemination of 3Rs information through NICEATM workshops and webinars

Dr. Bunton, second discussant, said from a strategic perspective she was thrilled to see the changes described by Dr. Casey and considered them in the right direction and appropriate for NICEATM. She felt the changes would help the participating agencies take ownership in the process, although there is no assurance that the agencies will act. She said there is clear momentum in the new direction, and praised Dr. Casey's work fostering a sense of urgency. She questioned how the SBIR grants would work with NICEATM. Dr. Casey explained how ideas from ICCVAM would be channeled through NICEATM to the NIEHS Division of Extramural Research and Training's SBIR grant program for development.

Dr. Olson, third discussant, noted the difficulty of judging NICEATM and ICCVAM initiatives independently, although it is clear that they are distinct entities. He felt the items identified in the description of new activities for NICEATM are clearly aligned with elements of the new operating paradigm. He was concerned with being able to disentangle NICEATM activities from ICCVAM activities. With the breadth of the

activities and the many options available, prioritization to gain the maximum return will be critical. Dr. Olson added that going forward, NICEATM activities would still be integral to coordinating the activities of ICCVAM, despite the other changes.

Dr. Casey said the relationship between NICEATM and ICCVAM should be synergistic. He felt that involving NICEATM in Tox21 would help promote a "validation, animal reduction" mindset. He said the impact on reduction in animal testing drives his thinking on every aspect of NICEATM and ICCVAM. Regarding NICEATM's support for ICCVAM, he cited the skin sensitization and oral-dermal projects as examples, as well as the 3T3 protocol as it replaces acute oral toxicity tests. Ultimately, he said, now is a great time for NICEATM and ICCVAM, and good results should be produced in the near future.

VI. Adverse Outcome Pathways (AOPs)

A. AOP Concept and Overview of ICCVAM Strategy for Skin Sensitization

Dr. Joanna Matheson, U.S. Consumer Product Safety Commission (CPSC), provided an overview of AOPs. She tied the concept back to the seminal 2007 National Research Council (NRC) publication, *Toxicity Testing in the 21st Century: A Vision and a Strategy*. AOP is a conceptual construct outlining the sequence of events from exposure through the adverse effect, targeting a mechanistic understanding of a chemical's effect at the molecular and cellular levels. An AOP should be definable and reasonable from a physiological and biochemical perspective and incorporate both toxicity and mode of action. It stems from a specific molecular initiating event and leads to a specific adverse outcome or apical endpoint. An AOP is designed to foster the ability to test multiple chemicals and extrapolate their effects, allowing the development of chemical categories, the identification of data gaps, and the development of integrated, tiered approaches to testing and assessment. Dr. Matheson described the process for developing an AOP and listed projects in the OECD AOP development program work plan, the first of which is skin sensitization. She illustrated the key steps in the skin sensitization AOP, tying the key AOP events to assays connected to them. She also listed current ICCVAM activities related to the development of AOPs, which include (1) the Electrophilic Allergen Screening Assay (EASA), (2) a NICEATM collaboration to develop and evaluate chemical structure-activity relationship (SAR) models to predict skin sensitization, (3) a NICEATM collaboration with industry scientists to develop an open-source Bayesian network as an operational framework for an integrated testing strategy (ITS), and (4) a NICEATM evaluation of various HTS assays in coordination with NIEHS Tox21 activities.

B. NICEATM Skin Sensitization Projects

Dr. Nicole Kleinstreuer, ILS, reported on current NICEATM projects related to the skin sensitization AOP. She described (1) collaborations to develop and evaluate chemical SAR models for predicting skin sensitization, (2) development of an open-source Bayesian network to predict skin sensitization, (3) coordination with the OECD AOP program for skin sensitization to guide development of an ITS, and (4) evaluation of HTS assays from ToxCast/Tox21 for relevance to skin sensitization.

She noted that NICEATM is supporting a number of efforts to create probabilistic frameworks for inference and ITS development, including the Bayesian network structure, which bases its topology on the skin sensitization AOP. She said well characterized AOPs such as skin sensitization are providing opportunities to use HTS data from ToxCast and Tox21. She reported that NICEATM is also working on a number of other AOP projects, including embryonic vascular disruption and developmental immunotoxicity.

C. SACATM Questions and Discussion

Dr. Black asked Dr. Kleinstreuer to elaborate on the data regarding coverage and sensitivity of the quantitative SAR models. Dr. Kleinstreuer said the advantage of the Bayesian approach is the ability to look for consistencies in predictivity based on biological knowledge, as opposed to anchoring to an animal test endpoint. She confirmed that the direction is to replace the Local Lymph Node Assay.

Dr. Cavagnaro asked whether there is weighting involved in mapping an assay to an AOP. Dr. Kleinstreuer confirmed that a Bayesian analysis allows weighting. She said other projects involve functional validation of assays mapped to steps in an AOP, and using models such as transgenic zebrafish and morpholinos to target specific signaling molecules to determine their contribution to an AOP. Chemical prioritization through ToxCast is also being done. Dr. Cavagnaro suggested that characterizing various chemicals relating to skin sensitization, would be good information to feed into these models. In response to Dr. Cavagnaro's question about the Jaworska paper on Bayesian testing (*Bayesian Integrated Testing Strategy to Assess Skin Sensitization Potency: from Theory to Practice,* Jaworska J, Dancik, Y, Kern P, Gerberick F, and Natsch A. *J Applied Tox* (Epub 2013 May 14), Dr. Kleinstreuer said that a more comprehensive and robust training set would be used improve predictions. Dr. Matheson noted that a presentation by Jaworska (2010, *http://media02.jhsph.edu/caat/hartungjaworska.mp4*) stated that testing beyond four assays appears not to be informative.

Dr. Toth, first discussant, praised ICCVAM for its transformation within a short time frame, but noted that it would be critical that the member agencies embrace their new

roles in setting priorities and directions. She said a coordinated effort to develop and define the AOPs would be essential for their acceptance and application in the regulatory environment, and any one agency or group alone would not be capable of covering the entire complexity of the AOPs. She felt ICCVAM should stay on target by defining the components and relationships within an AOP that provide regulatory utility, particularly with regard to the 3Rs. She noted that the AOP models are largely linear, which may limit their applicability in terms of risk assessment. The limits of extrapolation and application should be carefully defined, she pointed out, and the AOPs should be fully annotated. She expressed concern that although the AOPs are clearly a good way to organize information, they may drive or limit further research. She noted that the Bayesian approach, as opposed to the AOPs, is not an informational strategy but can make use of informational strategies and be used as a tool to identify a battery of alternative tests that will provide an optimal *in vitro* testing strategy. She felt it would be important for ICCVAM to develop subgroups to focus on particular AOPs and Bayesian network strategies that would fit needs of individual stakeholders, as opposed to trying to develop a "one size fits all" model. The Bayesian model would also need to have clearly defined limits of application or extrapolation. She added that under either approach it would be important to clearly identify who holds ownership of the systems and who is responsible for maintaining the databases and algorithms. She urged that determination of which AOPs are developed be a group effort among ICCVAM, the regulatory agencies, and other stakeholders.

Dr. Chapdelaine, second discussant, said that to effectively use AOPs, a coordinated effort would be necessary to evaluate the assays and methods being used so they are accepted by the industry as a whole, as well as by the regulatory agencies. She noted that ICCVAM should coordinate that effort. She said for each AOP, the key steps would need to be identified from the initiating event to the toxic effect or outcome, with skin sensitization providing a model with a good understanding of the key events in a specific AOP. ICCVAM should coordinate the review and validation of the proposed methods and ensure that the most appropriate methods move forward. She supported the AOP for skin sensitization. She felt the Bayesian ITS has an advantage of being adaptive with respect to new information because it can be used when not all of the data to generate a hypothesis are available. Prioritization of the AOPs should take into account which ones are most needed to address the 3Rs and which have the most chance for success. She noted that for some adverse effects, AOPs might not be feasible. ICCVAM should encourage both members of industry and academia to develop alternative testing methods, and urge the regulatory agencies to become actively involved in the process.

Dr. Black, third discussant, said that as a new participant, she was trying to understand how ICCVAM prioritizes its efforts. She noted that currently there is no clear path

forward for the use of alternative assays in drug development. She wondered whether there were some "low-hanging fruit" assays that could be instituted with buy-in from the applicable regulatory agencies, particularly in cardiovascular risk assessment. She urged consideration of national and global public health concerns for helping to identify the most important needs among the regulatory agencies. She said ICCVAM, NICEATM, the FDA and the other agencies should adopt a global perspective for alternatives, as opposed to a single-assay orientation.

Dr. Ochoa supported Dr. Black's point on prioritization, calling for the development of metrics that would allow definition of which projects should take priority. Dr. Cavagnaro mentioned the importance of validation against human relevance. She added that all drugs that have been pulled from the market due to toxicities, as well as many failed drugs, should be in the databases such as Tox21, to provide a human correlate for toxicity. Dr. Black noted that several failed drugs are in the ToxCast database. She added that not knowing how disposition of a drug or toxicant affects accumulation in the body, especially from repeated exposures, is a barrier to moving away from animal studies, as *in vitro*, cell-based studies may not yield that information. She said she looks forward to being educated on how any of the models or any of the AOPs takes that issue into consideration.

VII. Tox21 Update

A. Presentation

Dr. Raymond Tice, Chief of the NTP Biomolecular Screening Branch, briefed SACATM on the status of Tox21, in a presentation titled *Tox21: A U.S. Federal Collaboration to Improve the Human Hazard Characterization of Chemicals*. He described the Tox21 community, provided a history of the formation of the collaboration, outlined its goals, and illustrated its organization, including points of contact within each of the collaborating agencies and details on the four Tox21 Working Groups.

He noted that Tox21 Phase I (2005-2010) involved proof of principle using ~2800 compounds screened across 77 biochemical- and cell-based assays, while Phase II (2011-2014) focuses on expanded screening of the Tox21 10K compound library in a set of nuclear receptor and stress response pathway cell-based assay. He described the types of assays and informatics methods being employed in the screening process. He provided several examples of recent and ongoing Tox21 projects, including the NIEHS – National Center for Advancing Translational Sciences – University of North Carolina at Chapel Hill Toxicogenomics Project, which evaluated human cell line variability in response by screening 1086 lymphoblastoid cell lines representing 9 racial groups for cytotoxicity caused by 179 chemicals. Tox 21 Phase III (started in 2013) will focus on improving biological coverage and relevance, using high-content assays and

high-throughput transcriptomics platforms and cell models that incorporate an increased ability to metabolize xenobiotics. Phase III will also involve increased use of *in silico* models and extrapolation models, expanded utilization of lower organism model systems, use of 3D tissue models, integration of the AOP concept, and expanded collaborations and networking. Among the expanded collaborations are several collaborative, stem cell-related projects, where the usefulness of induced pluripotent stem cell (iPSC)-derived differentiated cell populations (cardiomyocytes, neural cells) in toxicological testing are being evaluated. Another project is a request for grant applications for development of assays that can be adapted to a HTS format to evaluate the effects of toxicants on cell differentiation using multi-potent or pluripotent cells.

Dr. Tice delineated the many milestones reached by Tox21 through the course of its work, which include: (1) successfully characterizing the qHTS data structure and identifying the artifacts that lead to false results, (2) making progress in data analysis and in the development of tools for prioritization, (3) making all ToxCast and Tox21 Phase I data public, (4) making chemical libraries available to investigators to expand the breadth of toxicological information, (5) exchanging assays and data with other organizations/efforts (e.g., EU Joint Research Centre, Health Canada, Seurat, OpenTox), and (6) working with NICEATM and ICCVAM to evaluate the utility of Tox21 assay data for use by regulatory agencies.

He described the attributes necessary for the program's success: (1) robust scientific collaborations, (2) well-characterized chemical libraries, (3) well-characterized assays in terms of reliability and relevance, with broad biological coverage, (4) incorporation of xenobiotic metabolism into *in vitro* assays, (5) informatic pipelines/tools that integrate and mine diverse data streams, (6) understanding the relationships between pathways and disease in humans and animal models, (7) making all data public, and (8) outreach to the scientific community on the usefulness and limitations of Tox21 data. He characterized success for Tox21 as bringing: (1) test methods for toxicity testing that are scientifically sound and more economically efficient, (2) an increased ability to evaluate the large numbers of chemicals that currently lack adequate toxicological evaluation, (3) models for risk assessment that are more mechanistically based, and (4) reduction and/or replacement of animals in regulatory testing.

B. SACATM Questions and Discussion

Dr. Ochoa asked Dr. Tice to elaborate on the data illustrated in his slide regarding identification of untested chemicals with increased likelihood of *in vivo* reproductive toxicity. Dr. Tice explained that the graph depicted a prioritization tool to help determine which untested compounds should be tested, based on their *in vitro* activity pattern similarity to compounds known to be active in the rodent uterotrophic assay.

Dr. Wilson, first discussant, noted that well before the Tox21/ToxCast data would be used for regulatory acceptance, it would be used many other ways. Thus, he suggested that the various potential users should be queried as to how they would use the data, and then for Tox21 to modify the approaches based on user feedback. He approved of making the program's data public and suggested that it be put in one database. He recommended the formation of advisory groups for the various endpoints whose members have broad expertise. He also suggested prioritizing the approaches, starting with ones that are more reactivity-based. He recommended not moving away from apical endpoints, because regulators are still regulating based on the apical endpoints. He said one of the biggest challenges is acquiring the means to assess metabolism, bioavailability, and dose response. He was concerned about management of the testing and how to implement new approaches in industry, academia, and government. Looking at the rapid evolution of the field, he highlighted the importance of training scientists who generate and use the data.

Dr. Kahn, second discussant, found the update on Tox21 exciting. He said he had concerns about assessing metabolism and chronic exposures, and noted that it should be kept in mind how the Tox21 initiative ultimately affects human health. He felt there is some progress being made, despite tremendous challenges, with the prediction models still years away from being applied to humans.

Dr. Kastello, third discussant, considered the promise of Tox21--characterizing chemical hazards in humans by using automated methods and eliminating the need for animal testing--exciting; however, he felt few people actually understanding the scope and complexity of the enterprise. He suggested that use of smaller chemical libraries might facilitate validation by concentrating on SARs. He noted it would be important for Tox21 to include genetic diversity in the development of assays.

Dr. Tice said all Tox21 data would be in available in three different data repositories (PubChem, EPA's ACToR, and NTP's Chemical Effects in Biological Systems, or CEBS). He clarified that the program currently involves outside experts, particularly in data analysis, via data transfer agreements with various organizations. Regarding training, he said it is one of the biggest issues being faced both internally and externally. He noted the web-based dashboard being developed by EPA as one way to facilitate use by others of the complex data being generated. He agreed that addressing chronic exposures is a limitation of the program, but anticipated the current development and integration of 3D tissue models into Tox21 Phase III would help. He noted that there are three levels of compounds being used in Tox21; they include the 10K library, the EPA ToxCast 1,000 compounds, and subsets of compounds (generally on the order of 20 to 200) for testing in specific models of interest. He thanked the SACATM members for their comments, noting that Tox21 is an extraordinarily complex project relying on excellent researchers to push it forward.

VIII. Report from the International Workshop on Alternative Methods for *Leptospira* Vaccine Potency Testing

A. Presentation

By telephone, Dr. Geetha Srinivas, U. S Department of Agriculture (USDA) Center for Veterinary Biologics (CVB), briefed SACATM on the *International Workshop on Alternative Methods for Leptospira Vaccine Potency Testing: State of the Science and the Way Forward* that was held at the CVB in Ames, Iowa on September 19-21, 2012.

The workshop brought together more than 80 international scientific experts from government, industry, and academia to review recent advances in science and technology and available methods and approaches for *Leptospira* vaccine potency testing. The main focus of the workshop was on methods and approaches that are more humane, use fewer or no animals, and would provide improved accuracy, efficiency, and worker safety. Its goal was to develop a strategy to achieve global acceptance and implementation of scientifically valid alternative methods.

Dr. Srinivas provided a brief overview of the work being conducted at the CVB to reduce animal use in development of *Leptospira* vaccines. She reviewed leptospirosis disease, including its health risks and financial impact, and described the state of regulatory oversight related to potency tests for vaccines for the disease. The codified potency test requires at least 40 animals. She described the vaccine disposition and the validity requirements for the codified potency test and pointed out that there are several disadvantages of the hamster potency assay, including the issue of animal welfare. Animal welfare was one of the motivations for the USDA's development of an ELISA-based test, which is advantageous in terms of animal welfare, cost, time, labor required and potential personnel exposures.

At the workshop communication between the CVB and vaccine manufacturers was identified as essential. Vaccine manufacturers were encouraged to initiate or continue product-specific validation with ELISAs. The CVB was encouraged to re-examine the necessity of back-titration animals in the hamster challenge assay. Workshop attendees strongly supported international harmonization of alternative potency methods. Dr. Srinivas described CVB studies to examine the impact of over-challenge on sub-potent serial distribution as part of an effort to reduce the use of back-titration animals.

B. SACATM Discussion

Dr. Ochoa supported the CVB's efforts to replace the present system of *Leptospira* vaccine potency testing. He questioned the possibility of significant reduction in animal use through the model Dr. Srinivas had described. He noted that there are alternatives

to LD_{50} testing, and it should not be necessary to sacrifice 10 animals in every group. He said it should be reconsidered whether so many tests need to be conducted just to prove that a particular lot is efficacious. He asked about the possibility of an intermediate approach to vaccine potency testing that might use fewer animals. Dr. Srinivas explained that her group is reviewing the LD_{50} requirements with the goal of eliminating it, which would reduce animal use by 50%. She reiterated that the workshop had resulted in improved communication between industry groups and regulators.

IX. Report from the International Workshop on Alternatives to the Murine Histamine Sensitization Test (HIST) for Acellular Pertussis Vaccines

A. Presentation

Dr. Richard McFarland, Office of Cellular, Tissue, and Gene Therapies, U.S. FDA, reported on the *International Workshop on Alternatives to the Murine Histamine Sensitization Test (HIST) for Acellular Pertussis Vaccines: State of the Science and the Path Forward*. The workshop was held November 28-29, 2012, at the Natcher Conference Center at NIH in Bethesda, MD.

The workshop provided a forum to discuss and review the *in vitro* protocols and available data from the International Working Group on Alternatives to HIST. The workshop participants reviewed additional new methods and approaches for acellular pertussis (aP) vaccine safety testing that should improve test accuracy, precision, and efficiency while also reducing or replacing the use of animals in vaccine safety testing. Finally, the workshop participants discussed the path toward global validation, acceptance, and implementation of scientifically valid alternative methods for aP vaccines.

Dr. McFarland briefly reviewed information on pertussis, which is the etiologic agent of whooping cough, and current *in vivo* pertussis vaccine safety testing, which requires large numbers of animals that experience unrelieved pain and distress. He related that pertussis vaccines had been identified as one of the highest priorities for human vaccines research, leading to an earlier ICCVAM-NICEATM Vaccine Potency and Safety Testing Workshop in 2010, as well as other international meetings on alternatives to HIST vaccine safety testing. More than 40 experts from 11 countries, representing government, academia, and industry attended the 2012 workshop. The workshop report has been submitted to *Biologicals* for publication, which is expected in October 2013.

Dr. McFarland reviewed the workshop's objectives and the state of the science, including pertussis vaccine adjuvant desorption methods. He also described the International Working Group on Alternatives to HIST 2012 study, which included 12 laboratories studying 7 vaccines from 3 manufacturers. Both biochemical and cell-based assays were evaluated. The group recommended development of specific reagents that would reduce variability. The group will next study the Chinese hamster ovary (CHO) cell assay.

At the 2012 workshop, several conclusions were reached: (1) no single method discussed was sufficiently developed for harmonized validation studies at this time; (2) a single method protocol is not applicable to all aP vaccines; (3) a goal is to identify a general assay/testing strategy to accommodate differences between vaccines (e.g., adjuvant types); (4) assays selected for further optimization/testing should be robust, sensitive, reproducible, easy to initiate, and cost effective when compared to HIST; and (5) the importance of harmonization was recognized for the next collaborative study, which is scheduled for 2013.

Participants recommended the design and methods for a small collaborative study to be pursued in 2013. Dr. McFarland noted that there may be a satellite meeting on the topic in Prague in 2014, and that the next international workshop on the subject is scheduled for 2014 in London. He pointed out that it is anticipated to take 1-5 years to complete the project.

B. SACATM Discussion

Dr. Black asked if there were any human data showing that the mouse HIST assay is an effective predictor of immunogenicity, thus leading to the need for the assay. Dr. McFarland replied that the need for the HIST assay in the whole cell vaccines was identified early on. The pertussis vaccine is part of the diphtheria/pertussis/tetanus (DPT) vaccination given to healthy young children. The risk/benefit ratio for adverse responses in vaccines is different from that in conventional pharmaceuticals given to people with diseases. Dr. Black asked if it were possible to do a biochemical fingerprinting of the protein found in the vaccine to understand its confirmation well enough to predict its immunogenic stability. Dr. McFarland said it is not possible now, but work on that issue using the CHO assay is underway.

X. Updates on International Collaborations

A. Presentation

Dr. Casey reported to SACATM on ICATM activities. He noted that ICCVAM recognizes that it is essential to be in a position to accept recommendations originating

from ECVAM. He showed the ECVAM alternative methods pipeline, noting it contains very few methods, even though ECVAM is the largest validation organization in the world and Europe has banned animal testing. Dr. Casey noted that, similarly, ICCVAM does not have a backlog of alternative methods to be addressed.

B. SACATM Discussion

Dr. Kastello asked Dr. Casey to speculate on why the pipeline for alternative animal test methods is so thin. Dr. Casey replied that there is not much incentive or potential profit in the area of alternative testing, and given the time and millions of dollars spent, there have not been any truly revolutionary alternative methods developed. After working to to replace animal tests for the past 15-20 years, there is now realization that there is never going to be a one-to-one replacement, so different approaches must be tried. Other approaches, such as Tox21 and computational chemistry, are needed and thus the impetus behind the change in NICEATM's activities.

XI. Adjournment

Drs. Bucher and White thanked SACATM for their participation in the meeting. Dr. Niemi adjourned the meeting at 5:00 PM on September 24, 2013.