

**Summary Minutes
SACATM Meeting
September 5-6, 2012
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 2012 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
ACAW	American College of Animal Welfare
ACD	allergic contact dermatitis
AOP	adverse outcome pathway
AR	androgen receptor
AVMA	American Veterinary Medical Association
BoNT	botulinum neurotoxin
BCOP	Bovine Corneal Opacity and Permeability
CERI	Chemical Evaluation and Research Institute, Japan
CPSC	Consumer Product Safety Commission
DABT	Diplomate of the American Board of Toxicology
DARPA	Defense Advanced Research Projects Agency
DMSO	dimethylsulfoxide
DPRA	Direct Peptide Reactivity Assay
EASA	Electrophilic Allergen Screening Assay
EDSTAC	Endocrine Disruptor Screening and Testing Advisory Committee
EEP	ECVAM Expert Pool
EDSP	Endocrine Disruptor Screening Program
EPA	U.S. Environmental Protection Agency
ER	estrogen receptor
ESAC	ECVAM Scientific Advisory Committee
EU	European Union
EURL/ECVAM	The European Union Reference Laboratory for Alternatives to Animal Testing
FDA	U.S. Food and Drug Administration
HIST	Histamine Sensitization Test
HTS	high throughput screening
IACUC	Institutional Animal Care and Use Committee
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ICATM	International Cooperation on Alternative Test Methods
ICE	Isolated Chicken Eye
ILS	Integrated Laboratory Systems, Inc.
IRE	Isolated Rabbit Eye
IWG	Implementation Working Group
JaCVAM	Japanese Center for the Validation of Alternative Methods
KoCVAM	Korean Center for the Validation of Alternative Methods
LLNA	Local Lymph Node Assay
MOU	Memorandum of Understanding
NAS	National Academy of Sciences
NCATS	National Center for Advancing Translational Sciences
NICEATM	NTP Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences
NINDS	National Institute of Neurological Disorders and Stroke
NIOSH	National Institute for Occupational Safety and Health

NIH	National Institutes of Health
NTP	National Toxicology Program
OECD	Organisation for Economic Cooperation and Development
OMB	Office of Management and Budget
PCRM	Physicians Committee for Responsible Medicine
PETA	People for the Ethical Treatment of Animals
PhRMA	Pharmaceutical Research and Manufacturers of America
qHTS	quantitative high throughput screening
RFA	Request for Application
RUC	rat uterine cytosol
SACATM	Scientific Advisory Committee on Alternative Toxicological Methods
SBIR	Small Business Innovative Research
STTA	Stably Transfected Human Estrogen Receptor Transcriptional Activation
STTR	Small Business Technology Transfer
TA	transcriptional activation
USDA	United States Department of Agriculture
ZET	Zebrafish Embryo Test

II. Attendance

SACATM met on September 5-6, 2012, at the National Institute of Environmental Health Sciences (NIEHS) in Research Triangle Park, North Carolina. The following individuals attended the meeting:

SACATM Members

Tracie Bunton, DVM, PhD, Eicarte, LLC
Joy Cavagnaro, PhD, DABT, RAC, ATS, RAPS, AccessBIO, L.C.
Joan Chapdelaine, PhD, Calvert Laboratories
Eugene Elmore, PhD, University of California, Irvine
Mark Evans, DVM, PhD, Pfizer
Steven R. Hansen, DVM, MS, MBA, DABT, ABVT, American Society for the Prevention of Cruelty to Animals
Gwendolyn McCormick, DVM, MS, DACLAM, BoehringerIngelheim
Steven Niemi, DVM, DACLAM, Massachusetts General Hospital (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
Marilyn Wind, PhD, Consultant

International Liaison Representatives

PatricAmcoff, PhD, The European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM)
Ki Hwan Choi, PhD, Korean Center for the Validation of Alternative Methods (KoCVAM)
CheaHyung Lim, DVM, KoCVAM
Hajime Kojima, PhD, Japanese Center for the Validation of Alternative Methods (JaCVAM)
Tim Singer, PhD, Health Canada

ICCVAM Primary Representatives

Steve Hwang, PhD, DOT (by teleconference)

Abigail Jacobs, PhD, U.S. Food and Drug Administration (FDA)
Jodie Kulpa-Eddy, DVM, U.S. Department of Agriculture (USDA), ICCVAM Chair
Anna Lowit, PhD, U.S. Environmental Protection Agency (EPA) (by teleconference)
Joanna Matheson, PhD, Consumer Product Safety Commission (CPSC), ICCVAM Vice-Chair
MoizMumtaz, PhD, Agency for Toxic Substances and Disease Registry (ATSDR)
Paul Nicolaysen, VMD, National Institute for Occupational Safety and Health (NIOSH)
RADM William Stokes, DVM, DACLAM, NICEATM Director, NIEHS

Other ICCVAM Representatives

Richard McFarland, MD, PhD, FDA/Center for Biologics Evaluation and Research
Stephanie Padilla, PhD, EPA
Geoffrey Patton, PhD, FDA/Center for Food Safety and Applied Nutrition

Invited Speakers

Mary Manibusan, PhD, EPA (by telephone)
Daniel Shaughnessy, PhD, NIEHS
Margaret Sutherland, PhD, NIH/National Institute of Neurological Disorders and Stroke (NINDS)

NIEHS/NIH Staff

John Bucher, PhD, NTP	Debbie McCarley
Warren Casey, PhD, DABT	Raymond Tice, PhD
Melissa Gentry	Mary Wolfe, PhD
Robbin Guy	Lori White, PhD, PMP, SACATM
Robin Mackar	Designated Federal Officer

Other Federal Agencies

Itai Chipinda, PhD, DABT, NIOSH
David Lehmann, PhD, EPA
Paul Siegel, PhD, NIOSH

Bridport Services, LLC

Ernie Hood, MA

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

David Allen, PhD	Steven Morefield, MD
Thomas Burns, MS	Michael Paris
Patricia Ceger, MS	Lori Rinckel, PhD
Vivian Doelling, PhD	Catherine Sprankle
Jonathan Hamm, PhD	Judy Strickland, PhD, DABT
Nelson Johnson	James Truax, MA
Linda Litchfield	Linda Wilson

Public

Patricia Bishop, People for the Ethical Treatment of Animals (PETA) and Physicians
Committee for Responsible Medicine (PCRM) (by telephone)
Jeffrey Brown, PETA (by telephone)
Maureen Bunger, PhD
Marcus Jackson, ILS
Joseph Manuppello, PETA and PCRM (by telephone)

September 5, 2012

III. Welcome and Opening Remarks

SACATM Chair Dr. Steven Niemi called the meeting to order at 8:30 AM. All in attendance introduced themselves. Dr. Niemi welcomed the new SACATM members, Drs. Tracie Bunton, Joan Chapdelaine, Mark Evans, and Marilyn Wind.

NTP Associate Director Dr. John Bucher welcomed everyone to the meeting on behalf of NIEHS/NTP Director Dr. Linda Birnbaum. He welcomed the new SACATM members, the ICCVAM agency representatives in attendance, and the International Cooperation on Alternative Test Methods (ICATM) members. He noted that over the course of the meeting there would be much progress to review and many new ideas to preview. He provided some historical background about ICCVAM and the alternative methods field in general, which he said has matured over the past 15 years. He noted the evolution of new technologies in the field and new approaches such as performance standards and batteries of tests. Part of the maturation of the field has also been the change from competition to cooperation internationally, particularly with the advent of ICATM, as international partners seek to integrate and harmonize alternative methods approaches. He recognized the retiring SACATM members, Drs. Eugene Elmore, Steven Hansen, and Gwendolyn McCormick, and presented them certificates of appreciation.

ICCVAM Chair Dr. Jodie Kulpa-Eddy welcomed SACATM and expressed ICCVAM's appreciation for the time and effort put forth by SACATM to provide input to ICCVAM. She also welcomed the ICCVAM agency representatives in attendance.

Dr. Niemi acknowledged and welcomed the international representatives in attendance, Drs. Ki Hwan Choi (KoCVAM), Tim Singer (Health Canada), Patric Amcoff (EURL ECVAM), and Hajimi Kojima (JaCVAM). Designated Federal Officer Dr. Lori White read the conflict of interest statement for SACATM.

IV. Welcome and NICEATM-ICCVAM Update

NICEATM Director and ICCVAM Executive Director Dr. William Stokes updated SACATM on the groups' activities and progress over the past year. He reviewed the NICEATM-ICCVAM Five-Year Plan for 2008-2012, including the strategic directions and priorities described in the document. In that context, he related recent NICEATM and ICCVAM progress in a variety of areas. He noted that ICCVAM had produced two biennial reports during the period, as per its statutory mandate, that the number of adopted and available alternative test methods had tripled in the past five years, and that since 1999, 58 alternative test methods had been adopted, including 36 *in vitro* methods and 22 that involve reduced animal use and/or avoidance or reduction of pain and distress. He reported progress in development of alternative methods for toxicity testing, with replacement of the acute "6-pack" tests being a priority. He noted that there are now 26 alternative test methods available in that area, with significant advances in each of the 3Rs.

Alternative test methods have also become available for phototoxicity, dermal absorption, pyrogenicity, genetic toxicity, and endocrine disruption. In the area of biologics and vaccine testing, which involve several agencies in the Federal government, 14 alternative test methods have been adopted since 1999, and it is currently estimated that 50% of vaccines do not require animals for lot release potency testing.

Dr. Stokes updated SACATM on the status of several alternative endocrine disruptor chemical screening assays, ocular toxicity test methods, and skin sensitization test methods, which now incorporate the use of adverse outcome pathways and an integrated testing and decision strategy, both of which are recent developments in the alternative test method arena. He also described activities involving the development of acute systemic toxicity test methods.

NICEATM-ICCVAM has conducted and published the proceedings of two international workshops recently; a 2010 workshop on vaccine potency and safety testing, and a 2011 workshop on alternative methods for human and veterinary rabies vaccine testing. Later in September 2012, NICEATM-ICCVAM (in collaboration with ICATM partners) will conduct a workshop on alternative methods for *Leptospira* vaccine potency testing, and in November 2012, NICEATM-ICCVAM and ICATM will conduct an international workshop on alternatives to the murine histamine sensitization test for acellular pertussis vaccines.

Dr. Stokes reported on the status of the 2011 pyrogen test method nomination and the 2011 botulinum neurotoxin (BoNT) test method nomination, both of which have involved ICCVAM Working Groups.

Recent outreach activities conducted by NICEATM-ICCVAM have included participation at the 8th World Congress on Alternatives and Animal Use in the Life Sciences (August, 2011, Montreal), the Society of Toxicology Annual Meeting (March, 2012, San Francisco), and presentations at several other workshops, lecture series, and ICATM partners' scientific advisory committee meetings.

V. Regulatory Acceptance and Availability of ICCVAM-Recommended Alternative Test Methods

A. Presentation

Dr. Stokes briefed SACATM on recent regulatory acceptance and availability of ICCVAM-recommended alternative test methods.

In February 2012, Federal agencies accepted ICCVAM recommendations for use of the LLNA for potency determination. The assay can now be used instead of guinea pig maximization tests (GPMT) to assess potency of sensitizing chemicals, with a 33% reduction in animal use as well as avoidance of pain and distress in the animals that are used.

ICCVAM has recommended the BG1Luc ER TA (LUMI-CELL[®]) test method to identify human endocrine receptor agonist/antagonist activity of chemicals. Dr. Stokes related positive responses from the US EPA and CPSC, noting that reduction in animal use would result from the EPA's acceptance of the method for the Endocrine Disruptor Screening Program (EDSP), since a negative result in the Tier I test battery would be considered sufficient to preclude Tier II animal tests. Also, the test method has resulted in a new Organisation for Economic Cooperation and Development (OECD) Test Guideline (TG 457), which is expected to be formally adopted by OECD in September 2012.

ICCVAM recommendations to refine ocular safety testing have also progressed, including adoption by US agencies in 2011 and an updated OECD Test Guideline (TG 405), which is also expected to be formally adopted by OECD in September 2012. In October 2011, OECD adopted (Guidance Document 160) the ICCVAM-recommended Bovine Corneal Opacity and Permeability (BCOP) and Isolated Chicken Eye (ICE) ocular safety test methods.

B. SACATM Discussion

Dr. Daniel Wilson, first discussant, said his comments would address how to improve regulatory acceptance of alternative methods. He focused on four main areas: enhanced communication, enhanced outreach, enhanced tracking, and harmonization.

To enhance communication, he suggested that the existing vehicles described by Dr. Stokes be examined and challenged to see if they can be improved, with maximum use of electronic tools in today's environment of restricted travel. He endorsed an annual session at the SOT meeting to outline new Federal guidelines promoting animal welfare. Also, he suggested establishing community of practice or user groups, citing the success of similar efforts related to Tox21/ToxCast.

He proposed the establishment of a concise matrix of approved methods, to include all relevant information regarding worldwide regulatory acceptance and much more comprehensive information for potential users or developers of new test methods. Also, tracking information should be included as a way to gauge the success of a new method.

To enhance outreach, he suggested that the USDA include consideration of alternative methods implementation in their annual audits of laboratories using animals, that Association for Assessment and Accreditation of Laboratory Animal Care become involved in similar fashion, and that questions in the area be included in the Diplomate of the American Board of Toxicology exam.

He noted that USDA had recently issued a positive statement in the **Federal Register** regarding animal use, and suggested that it would be useful for the other regulatory agencies to do the same, to help stakeholders understand the agencies' requirements and experiences.

Dr. Chapdelaine, second discussant, focused on the need to communicate to the users what the acceptable alternatives are. She noted that although they are aware of the methods, some users are hesitant because they are unsure that the regulatory agencies will accept the data generated by the alternative methods. She endorsed establishment of user groups and felt that the regulatory agencies need to proactively push for use of alternative methods, communicating their requirements clearly to the community.

Dr. McCormick, third discussant, was pleased to see that most of the agencies were planning to use websites, newsletters, and training modules to educate their staff about the available alternative methods. With current tight budgets, however, she also recommended that ICCVAM and the Federal agencies increase the use of webinars for 3Rs education. She noted that the American Veterinary Medical Association (AVMA) has a new specialty group, the American College of Animal Welfare (ACAW), which should help to raise awareness of the 3Rs. She encouraged establishment of ties with ACAW to include animal welfare material in health sciences curricula. She supported an effort to engage more effectively with stakeholders, including the public, for example by talking with civic groups to further their understanding of the ongoing need for use of animals in testing along with the tremendous strides in animal welfare that have already been achieved.

Dr. Stokes thanked the discussants for their constructive comments. In response to Dr. Wilson's suggestion, he said there had been a discussion with the ICATM partners about development of a template to distill the voluminous reports down to a one-to-two-page, concise summary that would include the types of information Dr. Wilson mentioned. Each of the ICATM members could use the template to post on their websites a summary of the methods they are

working on, which would provide potential users with more information as they consider use of alternative methods. In response to Dr. Chapdelaine's comments, he noted the example of the CPSC's proactive approach to recognizing acceptable methods and how they can be incorporated into their testing programs. He thanked Dr. McCormick for her ideas on how to improve communications, noting that partnering with interested organizations is often the best way to do so.

Dr. Kulpa-Eddy responded to Dr. Wilson's suggestion about USDA audits. She said that agency inspectors would be informed that as they inspect the facilities, they should be inquiring about whether the laboratories have considered using the new alternative methods that were recently approved. She noted that there are limitations, including the animal species covered by USDA.

Recognizing the achievements over the past five years that Dr. Stokes had presented, Dr. Hansen posed several questions that he thought should be of focus by ICCVAM: "What is the impact that we have had over those five years? What's the average number of animals that we used per submission five years ago, and what's the average number of animals that we are using today? If we can't answer these questions, how do we have any idea that we are on the right path?"

Dr. Stokes replied that it is very difficult to assess the number of total animals used, because many of the agencies do not submit data. He said a relative reduction of 50-60% could be achieved in the 6-pack of acute tests.

Dr. Toth said she was struck by the information Dr. Stokes had provided regarding the potential impact of replacing the 6-pack tests. She wondered if a similar analysis had been conducted regarding animal-based tests being used and accepted, but viewed as inefficient or inadequately predictive, and sub-optimal in terms of their outcomes. She asked if an approach had been taken in that area; to not necessarily focus on replacement of animal use but to take advantage of better information yielded by *in vitro* approaches. Dr. Stokes replied that that is one of NICEATM-ICCVAM's prioritization criteria.

In response to Dr. Hansen's questions, Dr. Chapdelaine suggested that one method would be to assess the number of guinea pigs used in testing, as submitted to USDA. She wondered whether the increasing use of the LLNA might have resulted in a significant decrease in the number of guinea pigs used. Dr. Kulpa-Eddy said that would be a possibility, but that when the agency gets such reports, there is a justification only with the Category E animals [Category E animals are those that are subjected to painful or distressful procedures without the use of anesthetics, analgesics, or tranquilizers. Withholding of anesthetics, analgesics, or tranquilizers can only be allowed if it is scientifically justified in writing and approved by the Institutional Animal Care and Use Committee (IACUC)]. If an animal is used for a particular test, but is not experiencing pain or distress, the agency may not be aware of the information in terms of compiling the total number of animals used. Also, the total animal numbers in the reports are subject to variation for a variety of reasons beyond 3Rs considerations.

Dr. Bucher said comments such as Dr. Hansen's had been heard many times throughout the course of SACATM meetings through the years. He asked the panel for its assessment of whether it would be more important to devote NICEATM-ICCVAM resources to communicating information about regulatory agency test acceptance than taking the next test method forward in the process.

Dr. Elmore acknowledged the difficulty of communicating large volumes of information concisely to the user community. He noted that in the U.S., agencies are not mandated to use and accept the data from new tests, although they may have formally accepted the method. Thus, users are sometimes unwilling to use new methods due to uncertainty and the resultant economic and marketplace constraints. He recommended that government team with industry to simplify communications and issue information that is readily understood, adding, "It's not always clear what the bottom line is." Consequently, that element often adds to reluctance on the part of industry to use alternative methods.

Dr. Ricardo Ochoa stressed the importance of metrics. He said he could not understand "the universe of what is happening" in terms of impact in the 3Rs due to the lack of data. He felt that the acceptance of alternative methods by users should be *driven* by the regulatory agencies, rather than their taking a passive approach. Dr. Bunton added that for those who are in a position of either having tests conducted or advising clients which tests to conduct, it is very important how the agencies are communicating which tests are going to be accepted.

Dr. McCormick suggested that ICCVAM survey IACUCs to acquire test and animal use data, as they would be in a position to have that information. Dr. Stokes said that was a good idea. He noted that a representative from USDA's Animal Welfare Information Center was recently designated to work with ICCVAM and should be able to help with communication by pushing more information out through the center's extensive Web resources.

VI. HTS/Tox21 Adaptation of the BG1 ER TA Test Method: Preliminary Assessment of Accuracy

A. Presentation

NICEATM Deputy Director Dr. Warren Casey briefed SACATM on the preliminary analysis of accuracy of the BG1 test method, as it has been adapted to the Tox21 high throughput screening (HTS) platform. The purpose of the assessment was to evaluate how well the method does in the high throughput setting, as well as to generate more data to help characterize the manual method—the HTS data on 10,000 compounds should help determine the applicability domain of any particular chemical.

Dr. Casey reviewed the history of the BG1 assay, noting that there are both estrogen receptor (ER) agonist (gain-of-function) and antagonist (loss-of-function) protocols, which is an important feature in terms of the 3Rs. He also presented background information on Tox21, which has as one of its major goals the development of *in vitro* assays to predict adverse outcomes in humans. The BG1 assay has been adapted from a 96-well, hand pipette procedure to a 1536-well, fully automated process.

Dr. Casey provided considerable technical details, including a comparison of the characteristics of the BG1 manual and qHTS procedures. In the qHTS study, 11,776 substances, of which 8,188 were unique, were tested three times each in BG1 agonist and antagonist assays. The list included most of the substances from the manual NICEATM validation study. Data from both methods were used to evaluate accuracy and concordance of the qHTS method relative to the validated BG1 manual method. Agonist concordance (61 substances) was 93%. There was also a very high level of accuracy in the agonists (34 substances; 27 positive, 7 negative). Antagonist concordance (71 substances) was 96%; accuracy was 100% (25 substances; 3 positive, 22 negative). Ultimately, Tox21 qHTS and manual testing produced almost identical results in terms of accuracy. The few discrepancies noted between Tox21 qHTS and the

manual method appeared to be primarily related to differences in the upper limit of testing concentrations.

Next steps in the project are to evaluate automated activity calling software for concordance with the manual method and to assess qHTS repeatability compared to the manual method. One recommendation in the ICCVAM Test Method Evaluation Report for the BG1 assay is to expand assessment of its ability to replace the rat uterine cytosol (RUC) assay through qHTS analysis. NICEATM has identified 142 compounds in the Tox21 10K library for which RUC ER binding data are also available. Similarly, ICCVAM has recommended that additional work be carried out to assess the possibility of the BG1 assay replacing the uterotrophic bioassay. NICEATM has identified 58 chemicals in the Tox 21 10K library for which rat uterotrophic data are also available.

B. Public Comment

Ms. Patricia Bishop provided comments from People for the Ethical Treatment of Animals (PETA) and the Physicians Committee for Responsible Medicine (PCRM) by telephone. Noting the “excessive” length of time it took for the BG1 LUMI-CELL[®] test method to be validated by ICCVAM, she said it “underscores the fact that current processes for validation of regulatory methods are inappropriate for many of the scientific tools being rapidly developed as part of the 21st century toxicity testing business.” She called for timely and appropriate validation methods that keep pace with the changing science and that meet regulatory needs, stating that ICCVAM’s current approach to validation does not seem capable of fulfilling that requirement. She noted that the BG1 has been presented as being at least as accurate as the Chemical Evaluation and Research Institute Stably Transfected Human ER Transcriptional Activation (CERI-STTA) assay, with some advantages, but it is an *in vitro* test being offered as an alternative to another *in vitro* test, thus offering no reduction in animal use. However, she noted, the BG1 method would reduce animal use if it was to replace the RUC ER binding assay and/or the rat uterotrophic bioassay. She said there is no evidence that ICCVAM plans to pursue investigation of either of the possibilities. She stated, “We urge ICCVAM to immediately initiate and complete further evaluation of the LUMI-CELL[®] assay as a priority replacement for both the rat cytosol ER binding and the rat uterotrophic assay.”

Dr. Casey noted that the inter-laboratory validation of the assay had actually started in 2007, although the assay was nominated in 2004, which Ms. Bishop had cited as the starting date of the validation process. He noted that the time for completion of the study in 2010 to peer review was less than one year, and that within another year the method had been adopted by OECD. Thus, he felt that ICCVAM had moved very quickly once it had control of the process.

C. SACATM Discussion

Dr. Elmore, first discussant, supported the expansion of the BG1 assay into HTS, but cautioned that the issues of bioinformatics and quality would be critical. He noted that endocrine disruptors are quite common, but that sometimes an adverse biologic effect had not been established, citing soy as an example. He said that the differences in responses between humans and other animals should be kept in mind, and that although the Tox21 platform is human cells, several agencies such as EPA also have interest in effects in animals. He also noted the issue of solubility of the compounds, and asked if any effort had been made to determine solubility and take it into account.

Dr. Wilson, second discussant, pointed out that some of the data that had been presented on the assay was being seen by SACATM for the first time. He noted that in the qHTS, the assay

had been an order of magnitude less sensitive, with high concordance and accuracy. In general the challenge with HTS assays is that they are often proprietary, with sophisticated, expensive robots, limiting reproducibility. He said that the dialogue about the nature and extent of validation that needs to occur and have some resolution should involve all of the stakeholders, with consideration of the performance standards that have been determined, regardless of whether the assay is low- or high throughput. He noted that it “would be very useful in the next couple of years to focus on looking at the constellation of HTS assays that are out there.” Dr. Wilson said there will soon be large datasets available comprising many thousands of chemicals having been run through several different HTS assays, and that they should be compared for their utility.

Dr. Cavagnaro, third discussant, focused on some of the terminology in use with the test methods—screening, acceptance, weight of evidence, and approvability, or the regulatory use of the methods. She said that she would particularly like a bit more consideration about what is meant by “weight of evidence.”

Responding to Dr. Elmore’s question about solubility, Dr. Casey said solubility was considered, aiming for a final concentration of 100 μM . Dr. Raymond Tice, NIEHS/DNTP confirmed Dr. Casey’s assessment, adding that there was a constant concentration of DMSO in each well, with no variability, including a control plate with DMSO only. He said that each of the 1536 wells has four columns that contain both negative and positive controls, and the data are normalized on a plate against the positive control response and the difference with the negative control response.

Responding to the question about viability, Dr. Casey said there were two signals: the signal itself, or a ratio that divides the signal by the viability, which sometimes yields non-intuitive results. He said they look manually to see where viability starts to drop off, and use that as the limit of what is considered to be good data. In terms of terminology, he said that prioritization is used frequently to help identify the most hazardous chemicals that need to be tested first; a top-down approach. He felt that SACATM is likely more focused on a more bottom-up approach, where a number of negative assays would preclude the need for a chemical to be tested in animals. The data analysis is completely different between those two approaches, he added. He noted that there is some effort being expended as part of Tox21 to look at a combined ER and AR assay. Dr. Tice related more details on that initiative.

Dr. Bucher addressed Dr. Cavagnaro’s question regarding weight of evidence. He said he prefers to think of the process as development of decision trees as opposed to weight of evidence assessments. The critical point is to be able to know when it is possible to stop evaluating, accepting that a compound is negative. Dr. Cavagnaro felt that the question about regulatory acceptance had not yet been adequately answered, and said that perhaps weight of evidence is more of a regulatory term in that context, with the decision tree informing the weight of evidence.

Dr. Stokes noted that one of the differences between prioritization and screening in terms of definitions is that in the context of ICCVAM, a screening test can be used for some kinds of decisions, but not the total decision on safety or hazard. He said the goal of these screening assays to eliminate that middle ground of uncertainty without having to run a more definitive test that uses animals.

Dr. Elmore said it should be remembered that an HTS assay is an acute assay, and that acute tests will not pick up some of the effects of long-term exposures.

VII. ICCVAM Test Method Nomination: Electrophilic Contact Allergen Identification Screening Assay

A. Presentation

Dr. Joanna Matheson, CPSC principle representative to ICCVAM, ICCVAM Vice-Chair, and Co-Chair of the ICCVAM Interagency Immunotoxicity Working Group, briefed SACATM on the nomination of the electrophilic allergen screening assay (EASA).

She briefly reviewed the NICEATM-ICCVAM test method nomination process and ICCVAM's five criteria for prioritization, along with reasons to evaluate the skin sensitization potential of chemicals and products and why products that cause allergic contact dermatitis (ACD) are regulated. She provided historical background regarding ICCVAM's activities related to ACD.

The EASA test method was nominated by Dr. Paul Siegel of NIOSH. It is an *in chemico* test method for screening ACD hazards, and was nominated for evaluation as a screening assay to identify contact allergens, and for inter-laboratory validation studies to determine the most appropriate decision criteria to maximize sensitivity and specificity of the assay. Dr. Matheson said that the assay could contribute to full replacement of animal testing for skin sensitization within the next year.

She presented information on the key events in the ACD adverse outcome pathway (AOP), which has become an increasingly important concept as it relates to application to integrated testing strategies. She related those key events in the ACD AOP to the various skin sensitization assays.

Relating the nomination to the five ICCVAM prioritization criteria, she noted that several of the US regulatory agencies need ACD test methods, and that dermal toxicity testing, including ACD testing, is a high priority area for ICCVAM. As an *in chemico* method, the assay uses no animals. It could be used as an alternative to the LLNA to test substances for human ACD hazard, and can also provide potency information. It has good sensitivity, specificity, and accuracy within the applicability domain, and could provide essential information to improve integrated testing and decision strategies for ACD hazard identification, potentially providing information for human health risk assessment. It is easy to perform, with rapid results and a low cost. It is also amenable to high throughput automation.

Dr. Matheson said the ICCVAM Interagency Immunotoxicity Working Group has reviewed the nomination and determined that it is of sufficient interest and applicability to warrant validation studies, and that it should have a high priority.

Dr. Siegel, joined by his NIOSH colleague Dr. Itai Chipinda, provided more details on the basic science and methodologies underlying the assay. He shared data on 55 chemicals that had tested positive as electrophiles in development of the assay. He noted that the method does not detect metals or prohaptens and that these are the major source of false negatives with the assay. He summarized the comparability of the assay with the Direct Peptide Reactivity Assay (DPRA) and LLNA assays, highlighting its potential utility as a preliminary screening assay.

B. Public Comment

Mr. Jeffrey Brown submitted comments on behalf of PETA and PCRM by telephone. Mr. Brown said that in general their organizations find the proposal acceptable given the potential usefulness and simplicity of the screening assay. However, the acceptability on condition that ICCVAM ensure that reference chemicals are chosen from existing databases of chemicals for

which LLNA data have already been compiled, and that reference chemical selection and validation study design is coordinated with currently ongoing validation of *in vitro* methods for detection of ACD hazards at ECVAM. He stressed that “under no circumstances should additional LLNA tests be conducted during EASA validation.”

Dr. Siegel said NIOSH has moved ahead and is working with ICCVAM and will use its guidance for choosing the chemicals and proceeding with the validation studies

C. SACATM Discussion

Dr. Chapdelaine, first discussant, agreed with the high priority given to the nomination by ICCVAM, noting that it has several advantages over currently used tests, particularly in that it does not use animals. Also, if either assay is positive, no animals would be needed at all. She also cited the assay’s rapidity and the absence of need for specialized training as distinct advantages, along with its requirement of standard laboratory equipment, which should aid its acceptance in the scientific community. She felt there was no reason the assay could not be used for systemic sensitizers as well as dermal, where there is much need for good models. She recommended that NICEATM-ICCVAM should encourage authors to nominate their assays and submit them for review.

Dr. Olson, second discussant, agreed with the need to give the assay extra attention. He questioned the goodness-of-fit metrics to substantiate the 10% and 30% probe depletion cut-offs offered in the logic tree, wondering how those criteria were derived. He asked how the result derived from this assay might differ from those of the Ames mutagenicity assay. He wondered how issues of solubility, molecular weight, log P or log D, and other determinants of dermal penetration might be integrated into scoring of EASA results. He also asked about the potential utility of variable pH in the solvent system, noting that the use of two pH values may contribute to making the assay somewhat more sensitive. He endorsed the idea of potentially using the assay in a tiered system. He was concerned about the possibility that the assay could stall out, citing the 50-60% completion with some of the test materials, and advised further investigation to determine a mechanistic reason for that happening. He noted that it should be a key responsibility of the Immunotoxicology Working Group and others to canvass the literature and look widely for methods that may be of utility, contacting developers and explaining that there is a “pull” for new methods. He said that this assay should be publicized as a model for effective recruitment of a test method by ICCVAM.

Dr. Bunton, third discussant, commended Drs. Matheson and Siegel for their enthusiasm for the assay and for having addressed the questions that had arisen during the written review. She wondered how the assay would fit in with others being developed or with other existing *in vitro* assays.

Dr. Evans, fourth discussant, felt the simplicity and rapidity of the assay are very strong recommendations for it. He advised caution regarding test article characterization, to ensure consistency. To encourage more nominations, he recommended that ICCVAM increase its presence with IACUCs.

Dr. Siegel said he agreed that the “stalling” phenomenon was perplexing and it would require additional mechanistic studies to understand the underlying chemistry. Dr. Matheson said DPRA assay developers have worked on a next generation assay with an additional step that would allow detection of prohaptens; she speculated that a similar solution might work for the EASA assay.

Dr. Stokes thanked SACATM for the comments and Dr. Siegel for bringing the assay forward, noting that it is just the type of innovative idea that NICEATM-ICCVAM likes to help further validate. He said the database of reference chemicals associated with the LLNA had grown considerably, and there should be no difficulty identifying the appropriate reference chemicals in this case. He added there would be coordination with the international partners to ensure consensus on selection of the reference chemicals.

Dr. Elmore asked whether kinetics could be used to assess potency. Dr. Siegel said that they could.

Dr. Niemi called for a vote on whether SACATM agreed with NICEATM's draft high priority for the nomination. Dr. Chapdelaine moved to agree with the priority; Dr. Wind seconded the motion. SACATM voted unanimously (12 yes, 0 no, 0 abstained) to accept the priority.

VIII. Federal Agency Research, Development, Translation, and Validation Activities Relevant to the NICEATM-ICCVAM Five-Year Plan

A. Presentation: NIH Microphysiological Systems Program

Dr. Margaret Sutherland, Program Director, National Institute of Neurological Disorders and Stroke (NINDS), provided SACATM with an overview of microphysiological systems being developed by NIH and the Defense Advanced Research Projects Agency (DARPA) in collaboration with the FDA. The National Center for Advancing Translational Sciences (NCATS) and the other collaborators are interested in improving drug development, both in terms of efficacy and drug toxicity prediction. The current system for drug and vaccine development is characterized by high attrition of drug candidates at every stage, due to efficacy failure, toxicity, or strategic considerations. Animal models are often imprecise in their correlation to human responses. Thus, an *in vitro* platform predictive of human toxicity, efficacy, absorption, distribution, metabolism and excretion is desired.

Dr. Sutherland depicted a generalized 3-D tissue model, which is comprised of common building blocks and bioengineered modules. The goal of these "organs-on-a-chip" is to be *in vitro* platforms that use human tissues that mimic the functions of specific organ systems in order to evaluate the efficacy and toxicity of medical interventions. Both DARPA and NIH have funded microphysiological systems research initiatives, with FDA on board to advise on regulatory requirements, validation, and qualification. The three agencies have also entered into a Memorandum of Understanding (MOU) ensuring that their programs will be highly coordinated.

The NIH initiative, which started with the Common Fund, involves 15 NIH Institutes and Centers, including NIEHS. The goal of the NIH investment of \$70 million over 5 years (involving two Requests for Application [RFAs]) is to develop microsystems with deeper complexity—that will be physiologically accurate, genetically diverse, and pathologically representative. The first RFA employs a UH2/UH3 mechanism (10 awards); the second a U18 mechanism (7 awards). The UH2 or Phase I grants will last two years and support research to develop physiologically relevant microsystems. Phase II, the UH3 grants lasting three years, will support research to integrate the microsystems developed during the UH2 phase. The U18 initiative is designed to support development of stem- and progenitor-derived cell resources to seed circulatory, endocrine, gastrointestinal, immune, integumentary, musculoskeletal, nervous (including eye), reproductive, respiratory, and urinary microsystems. Additional requirements include an intellectual property management plan in order to ensure eventual commercialization of the new technologies, a resource sharing plan, a milestones funding plan, and required participation in

the coordinated NIH and DARPA program and bi-annual workshops and quarterly conference calls.

Dr. Sutherland also illustrated the tissue chip program in terms of the parallel progressions expected in the NIH and DARPA programs, over the 5-year period. She emphasized that FDA participation is crucial to the success of the initiative by consulting at every phase of development of the systems to ensure that they will move regulatory science forward, including helping to select appropriate test compounds.

B. Presentation: SBIR/STTR Programs at NIEHS

Dr. Daniel Shaughnessy, Program Administrator, NIEHS, briefed SACATM on the status of small business funding programs at NIEHS. The programs were re-authorized by Congress in December 2011, with increased set-asides for Small Business Innovative Research (SBIR; 2.5%>2.6%) and Small Business Technology Transfer (STTR; 0.3%>0.35%) grants. SBIR set-aside will grow to 3.2% and STTR will grow to 0.45% by FY2017. Small Business Administration caps have also changed, with waivers now required for awards in excess of 50% over guidelines. Venture capital and other private equity firms are now allowed to participate, and agencies are now allowed to use up to 3% of SBIR set-aside funds for administrative purposes. SBIR/STTR grants typically follow a three-phase program: a feasibility study, full research/R&D, and a commercialization phase.

Dr. Shaughnessy outlined the major areas of emphasis for NIEHS, the most relevant to SACATM being the development of improved test systems for prioritization and safety. He noted that there are currently 7 SBIR grants in 3D human tissue culture systems, 5 in technology for animal toxicology studies, 9 in novel assays (2 STTR), 14 in sensors, 3 in biomonitoring technologies (1 STTR) and 4 in remediation (1 STTR). He described two of the 3D tissue culture models and four of the novel assays in more detail, as well as two of the FY12 contracts that are completing their Phase I work: a *3D Skin Model with Enhanced Sensitivity* and *Wide Field Volumetric Imaging of Cornea Injury for Earlier Humane Endpoints in Ocular Safety Tests*, both of which would be of interest to the 3Rs community.

With increased funding, it is likely that NIEHS will be soliciting more SBIRs through RFAs and Program Announcements. For example, last November the institute released a separate RFA on biomonitoring technologies, which will be funded very soon, supporting development of technologies allowing the detection of multiple analytes in a single sample.

He noted that applicants should look beyond the omnibus solicitation and watch the NIH Guide for announcements of SBIR RFAs and Program Announcements.

C. Presentation: Evolution from Traditional Data Requirements to Knowledge-based Requirements: EDSP21 Work Plan

Dr. Mary Manibusan, Director of the EPA Endocrine Disruptor Screening Program (EDSP), briefed SACATM by telephone on the evolution of the EDSP21 Work Plan. She outlined the program's history and background to date, including its mission and legislative mandates, which yield a combined universe of more than 10,000 chemicals to potentially be screened and tested. The 1998 Endocrine Disruptor Screening and Testing Advisory Committee (EDSCTAC) made key recommendations that formed the basis of the program, including establishment of a two-tiered screening and testing program.

Dr. Manibusan outlined the 11 tests that comprise the Tier 1 Screening Battery, including both *in vitro* and *in vivo* tests, and the 5 proposed Tier 2 tests, which are more targeted than the Tier 1 assays and will be used only when needed and appropriate.

Since the issuance of the final initial list of chemicals for Tier 1 test orders in 2009, EDSP is now beginning to receive those data for review—approximately 500 assays that will be going through a more extensive level of detailed review, and through the weight of evidence determination, it will be decided whether to advance those chemicals for Tier 2 testing. Given that pace of testing and screening, EDSP is exploring how future toxicological methods can streamline and accelerate the process, based on the vision provided in the 2007 NAS report, *Toxicity Testing in the 21st Century*. Tox21 moves away from the Tier 1 approach of requiring an entire battery of tests toward a more focused, targeted testing approach, only selecting studies necessary for regulatory decisions. This involves less reliance on animals and more reliance on *in vitro* and *in silico* models, more tailored generation of data, and is based on a firm understanding and integration of knowledge about toxicity pathways, exposure, and dosimetry.

The AOP concept is key to achieving those goals, in that it links the direct molecular initiating event to an adverse outcome at a level of biological assessment relevant to risk assessment. Dr. Manibusan illustrated the many data streams that inform the AOP.

Dr. Manibusan described the EDSP21 Work Plan, which was adopted in 2011. It is designed to maximize use of existing data while systematically and incrementally incorporating new tools and methodologies to advance understanding of key events in toxicity pathways. Prioritization of chemicals will be the focus in the near term (< 2 years), screening in the intermediate term (2-5 years) with high throughput *in vitro* assays replacing the current *in vitro* assays, and data replacement in the long term (>5 years), with high throughput *in vitro* studies and *in silico* studies completely replacing the current Tier 1 assays. To implement EDSP21, she said it would be important to ensure clarity of the programmatic goal, to find the application and regulatory decision contexts, to build a transparent strategy with a sound scientific basis, to determine scientific validity, and to ensure public outreach

D. SACATM Discussion

Dr. Olson, first discussant, said it was useful to have a broad overview of funded or to-be-funded activities in the alternative space. He noted that they all have the potential to contribute to the 3Rs and to the development of improved alternative safety testing methods, despite the fact that they may not start from a 3Rs perspective. He said the challenge would be to take best advantage of the many funded opportunities by identifying the methods with the greatest potential to contribute to future alternative safety assessment needs. There is a high probability for the NIH-funded opportunities to contribute to new and improved safety test methods that integrate alternative technologies, particularly the UH2/UH3 and UH18 initiatives. He supported continued focus by SACATM and ICCVAM on grants awarded from NIH intramural and extramural programs, while urging the development of a systematic means of capturing grantee/investigator findings to form the basis of a new generation of test methods.

He approved of the clear emphasis in NIH-FDA program promotional materials on continuation of the programs into 2013 and beyond, so that the funding would be available to sustain the programs over the long term, with their support for innovative and unconventional research. He found the SBIR program appealing, with its incorporation of business interests, noting that emphasis on commercial viability of new methods would focus on perceived likelihood of repeated use for fulfillment of regulatory or other safety test obligations. Regarding the EDSP

program, he felt that it emphasized the improved use of existing methods rather than the development of new methods.

Regarding the discussion question on how NICEATM and ICCVAM might help to facilitate the translation of the new research to standardized and validated test methods and models that might be useful for regulatory safety decisions, Dr. Olson related a number of specific ideas, which included (1) develop (via NICEATM or another group) a consistent focus and approach to review of research funded by the various mechanisms identified for discussion by SACATM (e.g., SBIR, NIH-FDA, etc.); (2) continue ICCVAM-sponsored workshops on select topics in safety assessment by alternative means; (3) partner with NIH, FDA, EPA, and other granting agencies to ensure that areas of research useful to ICCVAM (as part of the Five Year Plan) are recognized and incorporated into solicitations for funding applications; and (4) encourage via the SBIR program funding applications in target areas (toxicological endpoint, tissues/organs, etc.) that are recognized as needing alternative approaches in order to begin a move away from whole animal research conducted for safety evaluation.

Dr. Toth, second discussant, commended the presenters for exposing SACATM to “all of the incredible work that’s going on.” She noted there were two complementary, but perhaps parallel, funding trajectories; however, she felt that she had not heard that the point of the funding mechanisms was to provide an alternative to animal use as a central objective. With that omission, many valuable 3Rs ideas could be eliminated. She recommended that in new funding mechanisms, or at least in the evaluation criteria for existing mechanisms, alternatives are included. She also called for more overlap and integration across the various groups and funding mechanisms. She praised the ICCVAM workshops as a model of such communication. She hoped it could be determined what the regulatory community really needs in terms of funding mechanisms. She applauded the use of human tissue in some of the models.

Dr. Manibusan responded to the criticism that the use of alternatives was not specifically called for in EDSP21. She echoed those sentiments, noting that as they begin to evaluate the Tier 1 battery of information that was received from the initial list sent out in 2009, they will take that review before their Science Advisory Panel and attempt to optimize the battery, with an opportunity for the Panel to consider alternative methods.

Dr. Shaughnessy noted that although the guidelines for solicitations in the SBIR area do not specifically mention the 3Rs, many are addressed at *in vitro* testing, which speaks to those issues. He said ICCVAM and NICEATM are often mentioned by the small business applicants, so they are clearly reading the background information carefully and responding to 3Rs goals as well as commercial viability.

Dr. Abigail Jacobs clarified some aspects of the proposals. She noted they include many challenges, especially engineering challenges. She said the first uses would likely be for drug screening by drug companies. Some of the data would be from animal studies and some from human studies, which would aid predictivity. Some screens would undergo the process FDA calls “qualification,” which is detailed data reviews, but not formal validation as with ICCVAM.

Dr. Bucher agreed with Dr. Jacobs’ assessment in terms of the “multi-organs-on-a-chip” being applicable to drug development, but noted that NTP sees them as having potential uses in many other areas. He asked Dr. Sutherland to clarify her reference to validation of the processes, whether she was referring to toxicity or normal physiology. She replied that the human organs-on-a-chip would first be tested according to how well they represent the model organs. She noted there would be a great deal of data emerging from the studies, and that her group would be looking at a way to standardize and share it in a database.

Dr. Ochoa said, “this is the brave new world, this is where we’re going,” and that it is very forward-looking, though risky, to find solutions that go beyond those in use today. He approved of the animal-on-a-chip concept in that it addresses one of the limitations of *in vitro* studies. Noting that it is certainly possible that the project could fail, he asked what the criteria for success are, and what the milestones would be. He asked about the criteria for stopping a project, and who would have access to the technologies if they prove successful.

Dr. Sutherland noted that applicants were required to address intellectual property issues in the funding announcement. She said there should be a way to commercialize the technologies while still making them easily accessible to the community.

Dr. Wind said she was excited about some of the concepts presented, but struck by the fact that there had been no mention of ICCVAM. She wondered whether ICCVAM would actually be involved.

Dr. Stokes replied that fostering translation of such innovations into improved safety testing methods that have been standardized and validated is exactly what ICCVAM is seeking to do over the next five years. He said one of the goals of ICCVAM is to build bridges so the broader committee will be involved and engaged, as opposed to agencies working on things unilaterally. He added that right now that process is not systematic, and that the committee hopes to have a better structure for earlier and more frequent communication of this sort, to avoid “silos of excellence” and enhance sharing of information.

Dr. Jacobs noted that funding for the DARPA program was only to last five years. With the research being inherently risky, she said it is probably premature to discuss validation. The hope is that there will be proof of concept after five years.

Dr. Elmore approved of the concepts, but noted that they may be limited by the availability of tissue. He said the stem cell program might help the situation and agreed that proof of concept would be necessary before too much could be expected of the systems described. He noted that routes of exposure should be accounted for in the models, along with metabolites.

VIII. Draft NICEATM-ICCVAM Five-Year Plan (2013-2017)

A. Presentation

Dr. Joanna Matheson, ICCVAM principle representative from the Consumer Product Safety Commission (CPSC), ICCVAM Vice-Chair, and Chair of the Five-Year Plan (FYP) Subcommittee, briefed SACATM on the draft 2013-2017 FYP. The FYP emphasizes NICEATM and ICCVAM’s role in the ongoing transformation of safety testing, 3Rs progress made during implementation of the 2008-2012 FYP, and forthcoming opportunities for additional 3Rs progress. She noted that the FYP is relatively general in content and concentrates on strategic goals, while the Implementation Plan is more focused on concrete steps. The Implementation Plan is currently under development, with the hope that it will be completed by December 2012. She discussed in detail the four key strategic opportunities included in the FYP, which all contribute to the central goal of advancing innovative test methods of high scientific quality to protect and improve the health of people, animals, and the environment. To achieve that goal, NICEATM-ICCVAM will (1) promote the application and translation of innovative science and technology, (2) advance alternative test methods and testing strategies, (3) facilitate regulatory acceptance and use of alternative methods, and (4) develop and strengthen partnerships.

Ultimately, NICEATM-ICCVAM intends to replace and further reduce animal use wherever scientifically feasible, eliminate unrelieved pain and distress where and when animals must still

be used, and achieve continued and improved protection of public health, animal health, and the environment.

B. Public Comment

Mr. Joseph Manuppello commented on behalf of PETA and the PCRM by telephone. He said that in its FYP, ICCVAM “seems to consign itself to a minor role in advancing non-animal test methods, relying on its member agencies and European counterparts to take the lead.” He focused subsequent observations on the second strategic opportunity identified in the plan, advancing alternative test methods and testing strategies. He said that ICCVAM’s lack of progress in biologics and vaccines is “especially troubling.” He recommended replacing the LD₅₀ assay currently in use in botulinum toxin potency testing with validated *in vitro* tests. He recommended that the botulinum toxin workshop proposed by ICCVAM be given “the highest priority.” Mr. Manuppello expressed disappointment with ICCVAM’s ability to promote the use of humane endpoints for all challenge tests among its member agencies. In the area of acute toxicity testing, he noted that the evaluation of test methods and strategies that completely replace animal testing is absent from the plan. He also outlined several comparisons with practices and strategies by ECVAM. He described his organization’s stances with regard to ICCVAM’s plans in ocular toxicity testing, dermal toxicity testing, endocrine disruptor testing, and pyrogen testing. Mr. Manuppello urged SACATM to include representation from animal welfare organizations with relevant expertise in regulatory science issues.

C. SACATM Discussion

Dr. Hansen, first discussant, noted that the FYP does not seem to define animals or metrics, and that there is no reference to the 3Rs in its overarching statement. He suggested replacement language that incorporated a specific, measurable, meaningful target for reduction in animal use, with the other goals supporting it. He challenged the group “to do a much better job of writing a goal.”

Dr. Ochoa, second discussant, said he largely agreed with the points made by Dr. Hansen. He said that the goals were so broadly written as to be a restatement of the original goals of the organization, and lack specific outcome measures to address progress. He noted the use of the words “promote, advance, facilitate, and develop” without any metrics to assess achievement of the related goals. He said ICCVAM members should be committed to achieving hard, measurable goals through the use of better, more reliable metrics. He felt there is no sense currently of the universe of total animal use and ICCVAM’s influence upon it. He said NICEATM should have an office devoted to tracking metrics of animal use, and recommended a systematic review of practices and regulations that could be changed, such as the LD₅₀ no longer being required by the FDA. He noted that the mouse carcinogenicity assay is no longer used in Europe, and suggested that it could be a “low-hanging fruit” to be phased out in the US. Dr. Ochoa said the strategic goals should address the “spotty acceptance” of the alternative methods, with the perception that the regulatory agencies are reluctant to accept alternative methods. He considered that regulatory acceptance of methods another area where metrics would be helpful.

Dr. Olson said he felt the document should be titled as a *strategic plan*, to be followed by the Implementation Plan. He questioned the ordering of the strategic opportunities, suggesting it might be better for ICCVAM to focus more on its historical role of advancing alternative methods and test strategies, as well as facilitating regulatory acceptance, moving those items higher on ICCVAM’s priority list. He saw no need to add any more strategic opportunities to the plan. He suggested adding features called *Monitoring Progress* (which currently appears in conjunction

with goal #4) to all of the sections of the plan, to allow readers to see that there will be measurable metrics applied to each of the goals. He noted a thematic shift toward collaboration and flexibility, and urged that the intent to take advantage of the best emerging science in a nimble way be emphasized. He felt that the document does a credible job in detailing a broad area of research effort, including gaps.

Dr. Wind, third discussant, said the scope and focus of the plan were good, and approved of the acknowledgement of the rapidly changing technologies in the field, with the resultant need for flexibility. She noted that ICCVAM needs to be involved in many of the new technologies in order to be relevant when it comes to validation. Dr. Wind said that the plan's priorities are correct and she emphasized the need for collaborations with all organizations with the same goals working together to achieve them. She agreed with the research gaps identified in the plan, and called for an increase in communication and engagement with stakeholders.

Dr. Stokes said there had been much debate among the subcommittee on several of the topics just mentioned. He appreciated the emphasis on metrics, and said that progress had been made in that area, although the scorecards may need to be made more accessible on the website. He agreed that defining specific targets would be a good idea, along with implementation plans to meet the targets. He said ICCVAM was trying to maintain a balanced portfolio of near- and long-term activities.

Dr. Matheson liked the idea of adding a concrete target for reduction of animal use, but noted that it would be a hurdle to figure out how to measure that. She noted the documents are expected to change, so comments and suggestions would be welcome throughout the five-year period.

Dr. Cavagnaro urged further cataloging of progress by ICCVAM, as a central location for information about what is being done in the field. She also felt that ICCVAM could participate in crafting a decision strategy to promote regulatory acceptance.

Dr. Jacobs noted that it is the responsibility of the ICCVAM agency representatives to convey the information about what is going on the field back to their agencies.

Dr. Stokes said promoting regulatory acceptance starts at the very beginning of the process, including elements such as validation, study design, selection of reference chemicals, and test method protocols. Dr. Cavagnaro agreed with Dr. Stokes' assessment, but noted that the process needs to be promoted at every stage, with regulatory input throughout. She said acceptance is often confused with approval. Dr. Stokes noted there was an attempt in the report to clarify the difference between technical validation, which occurs early, and biological validation that confirms the fact that the results are biologically meaningful. He described "regulatory validation" as demonstrating that the assay is replicable at laboratories throughout the world, which is necessary for formal international regulatory acceptance.

Dr. Wilson noted the lack of acute toxicity data in the ToxRef database. He said that there could be significant animal savings by adding such data to it and other large databases that could then be easily data-mined to allow for read-across rather than animal testing.

Dr. Elmore suggested the USDA Animal Welfare Information Center might be asked to provide data on the number of different animals used, as they collect data from IACUCs. He said that while it was important for ICCVAM to continue to look at HTS and model systems, it would also be important to collect and analyze the large amounts of data involved, and see how well the

testing strategies integrate. He also encouraged ICCVAM to work more closely with industry to gather cross-platform information.

Dr. Stokes agreed with Dr. Elmore's point about the tremendous amounts of data generated by the high throughput methods, and said it was a major challenge to determine how to organize the data in the most meaningful way.

Dr. Niemi stated, "If we come to this meeting next year and there is no baseline of the number of animals used in something, then we may be wasting our time, pure and simple. If you want to continue to engage the enthusiasm and the energies of this advisory committee, we have to start counting so we know where we will be in three or four or five years versus where we are today, because we don't know where we are today." He said that quantification should be the major goal of any ICCVAM strategic plan. He suggested that a SACATM working group might be formed to help that effort.

Dr. Ochoa said that there should not be restrictions put upon the validation process in terms of use of animals, emphasizing the importance of scientific rigor. He said that it is desirable to avoid use of animals, but that scientific rigor in the process should take precedence.

September 6, 2012

IX. Workshops

A. Report and Recommendations from the NICEATM-ICCVAM International Workshop on Alternative Methods for Human and Veterinary Rabies Vaccine Testing

Dr. Richard McFarland, FDA, Co-chair of the ICCVAM Biologics Working Group, provided a report on the October 11-13, 2011 NICEATM-ICCVAM *International Workshop on Alternative Methods for Human and Veterinary Rabies Vaccine Testing*. He noted that the workshop had resulted from a major priority identified in the 2008-2012 ICCVAM FYP – vaccine potency and safety testing. The workshop involved multiple agencies and addressed the large number of animals used that experience significant unrelieved pain and distress.

Citing EU figures, he said the majority of animals used in testing were for production and quality control of medicines, biologics, and vaccines. More than 80 experts representing 14 countries attended the rabies workshop, held at the USDA Center for Veterinary Biologics in Ames, Iowa. Dr. McFarland delineated the objectives of the workshop, and described the recommendations that emerged in replacement, reduction, and refinement. He directed SACATM members to the meeting report, which was published in the journal *Biologicals* in August 2012.

B. Upcoming International Workshops: Alternative Methods for *Leptospira* Vaccine Potency Testing

Dr. Jodie Kulpa-Eddy, USDA principal representative to ICCVAM, Co-chair of the ICCVAM Biologics Working Group, and ICCVAM Chair, reported on the upcoming workshop devoted to *Leptospira* vaccine.

The *International Workshop on Alternative Methods for Leptospira Vaccine Potency Testing* is scheduled for September 19-21, 2012, at the USDA Center for Veterinary Biologics in Ames, Iowa. *Leptospira* vaccines were identified as a major ICCVAM priority at the September 2010 *International Workshop on Alternative Methods to Reduce, Refine, and Replace the Use of*

Animals in Vaccine Potency and Safety Testing: State of the Science and Future Directions. Replacement and refinement methods have been developed, but there have been delays in implementation, creating the need for a workshop to gather all of the stakeholders together in a non-regulatory setting. The meeting will be devoted to reviewing animal and public health perspectives, the state of the science, implementation issues, and future considerations to identify best practices for current and future integrated approaches to *Leptospira* vaccine potency testing. The workshop proceedings will be published in *Biologicals*.

C. Upcoming International Workshops: Alternatives to the Murine Histamine Sensitization Test (HIST) for Acellular Pertussis Vaccines

Dr. McFarland described the *Workshop on Alternatives to the Murine Histamine Sensitization Test (HIST) for Acellular Pertussis Vaccines*, which will take place November 28-29, 2012, at the NIH in Bethesda, MD. He provided some background information on pertussis, and noted that pertussis vaccine safety testing had also been identified as a high priority at the September 2010 workshop, because many lots are produced annually and HIST uses large numbers of laboratory animals that undergo significant unrelieved pain and distress. At the workshop, participants will review the usefulness and limitations of alternative *in vitro* test methods, review protocols and data generated by the International Working Group on Alternatives to HIST, and address validation and regulatory acceptance requirements. The workshop proceedings will be published in *Biologicals*.

D. SACATM Discussion

Dr. Cavagnaro, first discussant, felt that the rabies workshop had fulfilled its stated goals and objectives. She said there is “a clear opportunity for a metric to establish baseline animal use and assessing impacts.” She felt the recommendations to manufacturers *encouraging* use of alternative methods should be stronger, using the word *should* and requesting a time frame and a metric, with follow-up on implementation of recommendations for refinement, including tracking of several specific metrics. She suggested that in addition to the workshop summary, there should be an expectation for implementation, with clear recommendations covering a specific time frame. She described several opportunities for FDA to implement some of the recommendations. She felt the most important knowledge gap is the effort to address the best approach for adjuvanted vaccines. She mentioned several other *in vitro* approaches to assessing potency that should be considered, and expressed concern that sub-potent vaccine lots be detected accurately with new methods. She said workshop reports were welcome, but should include more concrete plans for deliverables, tracking, and providing reports to ICCVAM after a specified time has passed. She supported the concept of identifying regulatory acceptance requirements in all three workshop areas.

Dr. Evans, second discussant, said the overall organization of the workshops in relation to their stated goals and objectives was very good. He noted the common element of emerging private/public partnerships in the field, although he felt the private entities seemed to be under-represented. He wondered whether the technicians who run the tests are blinded, and if so, how, and whether sub-potent lots are ever inserted deliberately into assays. He noted test article and reagent characterizations become more important as assays move away from clinical endpoints to quantitative endpoints.

Dr. Hansen, third discussant, referred to a point made on one of Dr. McFarland's slides on the rabies workshop, “Manufacturers are encouraged to develop, validate, and implement *in vitro* antigen quantification methods to replace the NIH test.” Dr. Hansen felt the goal should have been more clearly defined, to delineate how to get buy-in from all stakeholders. He suggested

the goal should have been “to eliminate all animal use in five years.” He said there is a point in the process where ICCVAM could get directly involved to coordinate, particularly by building a business case that would appeal to the corporations. “ICCVAM should be the organization that’s dividing up all the pieces of the work, summing it up, counting it every year ... and making sure that at the end of five years we actually get to a point where we can have a celebration ... It could be a huge win, but ICCVAM’s got to take a leadership role in this,” he said. Dr. Hansen said this is a tremendous opportunity, where 80% of ICCVAM’s efforts should be devoted over the next five years.

Dr. Toth, fourth discussant, said the rabies workshop was extremely well organized and full of good information. She appreciated the approach at the workshop where problems with the current *in vivo* testing methods were described and compared with the various alternative methods, along with specific recommendations about which alternative methods should be pursued further. She also wished to see stronger definitions and strategies for humane endpoints. She concurred that regulatory acceptance is critical, and that having the manufacturers in the same room as the regulators is one of the strengths of these types of workshops. Regarding the future workshops, she recommended that additional emphasis be given to recognized problems with current testing methods, and how they might be resolved by alternative methods. She urged emphasis on the superiority and advantages of alternative methods in terms of improving assurance of product safety and efficacy.

Dr. Kulpa-Eddy addressed the request for involvement by more private entities in the workshops by pointing out that the slides she had shown were more oriented toward the working groups and organizing committees; the speakers at the workshops would be more diverse and would include industry representatives. She noted that competitive issues are sometimes a limiting factor in industry involvement. Regarding humane endpoints, she said there is a training video available for the rabies test to help provide additional information for technicians who would be running the test. Regarding why ICCVAM was not involved in the validation of the *Leptospira* vaccine test, she said it was because it is mainly a veterinary vaccine. She added that it is being brought forward to ICCVAM now in order to attain global reach for the assay by leveraging ICCVAM’s international partnerships.

Dr. McFarland addressed the suggestions regarding “stretch goals” and “business plans.” He said it is difficult to do either at the regulatory agencies given their primary mission, but that the concepts may be more useful for ICCVAM in its capacity as an umbrella organization. He agreed with the importance of follow-up beyond the workshops themselves. “What we don’t want to do is to have the effort for the workshop and then one to two, three, or four years down the road, see that nothing has happened in terms of reducing the animal use,” he said. Regarding the number of private entities involved, he noted that the vaccine industry is relatively small in terms of the number of producers, and that various acquisitions have reduced that number even further, for both the veterinary and human vaccines.

Dr. Stokes added that there were ten different companies that make rabies vaccine involved in the workshop, and that all of the human rabies vaccine in the U.S. is made by two companies, both of whom were there, along with all of the major animal rabies vaccine manufacturers. “This is some of the best industry participation that we’ve had,” he added. He said the workshops serve to help the companies understand whether the science is adequate to make a financial commitment to revising their applications to the regulatory authorities on product-specific validations. He agreed with the comments about monitoring progress and setting goals, although it would require involvement by industry stakeholders. Regarding the potential for earlier humane endpoints, he noted that it was necessary to ensure sufficient protection from

the disease by assessing the appropriate level of virulence, but said that there is potential to develop earlier indicators.

Dr. Toth said that given the limited resources available to ICCVAM, the workshops are an excellent activity.

Dr. Elmore inquired about the possibility of tracking the number of animals used with regard to the pertussis vaccine. Dr. McFarland said he was unaware of any data published, and that manufacturers are not required to provide that type of information in their applications. He said the topic would be broached at the upcoming workshop to see if the manufacturers are willing to provide at least “ballpark” baseline figures on animal use.

Dr. Niemi commended NIEHS for its support of the workshops, particularly since the topics of the upcoming meetings are outside of the institute’s normal bailiwick.

Drs. Stokes and Bucher recognized Dr. Kulpa-Eddy for her service to ICCVAM and SACATM, since this was her last SACATM meeting. Dr. Stokes presented her with a certificate of appreciation from ICCVAM, and Dr. Bucher presented her with a plaque from the National Toxicology Program.

X. Report from the SACATM Implementation Working Group

A. Presentation

Dr. Cavagnaro, Chair of the SACATM Implementation Working Group (IWG), presented the draft report from the IWG. The IWG was suggested at the 2011 SACATM meeting as an *ad hoc* working group to assess implementation of ICCVAM-recommended alternative methods. The IWG consisted of SACATM members Drs. Cavagnaro, Elmore, Hansen, Olson, and Wilson with assistance from Dr. White, SACATM Designated Federal Officer.

The IWG convened via eight teleconferences from March through August 2012, acting on its charge to assess implementation of ICCVAM-recommended alternative methods. Dr. Cavagnaro described current perceptions of a lack of clarity regarding acceptance, a lack of metrics and tracking, a need for regulatory champions, and limited oversight and accountability. Improved alignment with regulatory risk assessment strategies is needed, she added.

To begin to assess implementation of ICCVAM-recommended methods, the IWG developed two surveys, which were distributed to US companies and contract research organizations (CROs) that use alternatives. Questions were asked regarding submission of data generated by using ICCVAM-recommended alternatives. Dr. Cavagnaro provided details about the data generated from each survey and the IWG’s resulting recommendations, most of which concerned improvement in ICCVAM data collection regarding implementation by both industry and regulatory agencies. The group also recommended several specific actions to enhance implementation data collection, as well as improvement in communication regarding implementation with the regulatory agencies, specifically the EPA and the FDA.

Dr. White described the formation of the *ad hoc* working group under the SACATM charter, noting that any recommendations made by the group must be deliberated by the entire committee, with a vote on whether or not to accept the IWG’s draft report.

B. SACATM Discussion

Dr. Wind, first discussant, acknowledged that the IWG had addressed issues concerning ICCVAM that have arisen many times over the years. She said she was not surprised that the

in vitro cytotoxicity assay for picking starting doses was not used, as it was her impression that companies felt they already had sufficient data on hand for making those decisions. Dr. Wind said the IWG's recommendations would probably require the addition of at least one full-time person to ICCVAM's staff and given ICCVAM's limited resources, she wondered if that was practical. Dr. Wind observed that with some of the ICCVAM agencies, such as the CPSC, there would be no way to provide the desired data, because companies are not required to submit data as long as products are appropriately labeled. She suggested doing a pilot study, possibly starting with EPA, since EPA already collects and collates data on alternative methods.

Dr. Ochoa, second discussant, commended the IWG for its project, and said it is a good example of what SACATM should be going to evaluate ICCVAM. He suggested greater activity and interaction between SACATM meetings. He noted that even though the IWG's recommendations were tentative and the sample size was small, the report is a good attempt to address some of the questions concerning implementation. More data are necessary, he said, and suggested that Pharmaceutical Research and Manufacturers of America or other trade organizations could be good sources of information or assistance in collecting data. He observed that it appears clear from the surveys that there is much hesitancy by organizations to accept and use alternative methods, in that there may be misconceptions or misunderstandings about the acceptability of the methods by the regulatory agencies. He suggested the regulatory agencies look at how they communicate to industry and clarify their acceptance of alternative methods, perhaps using language such as *preferred* or *recommended* to communicate that they are actively seeking to increase use of alternative methods. He said the IWG's data should be rolled into a review of the ICCVAM FYP.

Dr. McCormick, third discussant, recommended that ICCVAM assess the "playing field" of all of the regulatory agencies that require animal testing and determine what they require. Then, ICCVAM could ask the agencies what criteria they would accept for use of alternative methods, and assess that information against what alternatives are already available. That would also help identify gaps where there are still areas of required animal testing, with no alternatives being developed. To totally replace animal use in the US, she said, it would take a mandate similar to the EU Directive, coming from Congress. Dr. Cavagnaro said the thought of absolutely not using animals is inconceivable, and not realistic in many cases. She thought it was unnecessary to go to the agencies because the guidelines are already known. Dr. McCormick said she was suggesting it would be useful to have a clear global idea of the areas where animal testing is required, what the criteria are, and where the greatest need for alternatives is.

Dr. Elmore recommended SACATM request quantitative information from directors of the regulatory agencies that use tests in order to determine the ability of the committee to fulfill its mandated duty to effectively advise ICCVAM on how to reduce animal use and increase the use of alternatives. He suggested requesting data on total number of tests done, the number of animals used, the number of alternative tests submitted for consideration, and whether or not they are stand-alone or weight-of-evidence. The agencies should be asked to respond within a period of time in order to establish a baseline. Dr. Elmore said asking the directors would help support the effort to extract the information, particularly from agencies with multiple divisions. He said it would be a win/win: for ICCVAM, with a way to document progress and how well it is meeting its mandate; for the organizations committed to replacing animal use; for people, with better science going forward to predict safety; and ultimately for animals as well.

Dr. Cavagnaro agreed, but said that the missing piece is the fact that alternatives are not being submitted to the agencies, as shown by the survey, because industry questions acceptance of

alternatives by the regulatory agencies. Thus, a survey of the agencies may not reflect true numbers.

Dr. Elmore said the agencies could not answer for the industries, as they can only describe what has been submitted to them. He said that for agencies, “we need to draw a line in the sand as to where we are going into this upcoming five-year period and see how well we progress over the years.”

Dr. Wilson said the IWG had tried to “get something out there as a way to provide a metric that would be something that we could use to attest to the success of ICCVAM.” He said the IWG had considered sending surveys to both the regulators and the regulated, and in the interest of practicality opted to survey the regulated industry only, just to get “a sense of where we are.” He recommended a pilot project with a regulated industry, perhaps with the EPA, to conduct more in-depth research over the course of the next year in order to get a deeper understanding of the current situation. He noted that people should not lose sight of ICCVAM’s many successes, many of which speak for themselves, as well as the very successful meetings and workshops the group has held.

Dr. Olson said the operation of the IWG demonstrates the willingness of SACATM members to take some time in the periods between meetings to focus on SACATM issues and to maintain some continuity of effort. He said the SACATM members are an underutilized resource and need some direction and cross talk from ICCVAM and the NTP on how to serve the purposes of ICCVAM in a better way. He noted that any number of areas could be developed with a working group strategy, while maintaining transparency, and recommended that SACATM consider establishing other working groups as an effective way to build communication and work together between annual meetings.

Dr. Bunton noted that a subtle change in language with the agencies could help promote the use of alternative test methods, recommending that they require justification when a submitter wants to use whole animal tests as opposed to the available alternative methods.

Dr. Chapdelaine commented that people doing studies must justify whole animal studies with the USDA. She agreed that it would be effective for all of the regulatory agencies to adopt that practice.

Dr. Niemi called for a vote on acceptance of the IWG’s report. Dr. Ochoa moved to accept the report. Dr. Chapdelaine seconded the motion. The vote was 10 yes, 0 no, 2 abstaining. Dr. Wind abstained, citing her earlier comments regarding ICCVAM’s limited resources and the lack of available data from agencies such as CPSC. Dr. McCormick abstained, expressing her concern about one of the report’s recommendations that required ICCVAM to establish a firm timeline for implementation, which she did not think was feasible.

Regarding the draft ICCVAM FYP, Dr. Niemi asked Dr. Matheson if there would be opportunity for SACATM to review further drafts, or if there would be a public comment period on revised drafts before it is finalized. Dr. Stokes noted that the timeline that Dr. Matheson had presented and that the committee had agreed upon included multiple public comment periods over the past year, and the intent is for the subcommittee to go back to work, finalize the document by the end of the year, and release the final five-year strategic plan in early 2013. Dr. Stokes said the plan is a living document and the subcommittee would continue to work on tactical plans with specific activities spelled out.

Dr. Cavagnaro suggested that the rabies initiative might be a suitable pilot for conducting a survey on how to collect animal use data, perhaps providing concrete numbers for SACATM's consideration next year. Dr. Niemi elaborated, noting that it could be a learning experience on what specifically to ask for, how to ask for it, and how those numbers might be collected and packaged for presentation at the next annual meeting or sooner.

Dr. Hansen asked for clarification on whether there would be further opportunity to comment on the FYP, and whether the idea that it will be a "living" document means that it changes. Dr. Stokes reiterated that the "strategic" FYP would be completed by the end of the year, with all comments having been taken into account. He said it was possible that SACATM could have further input with the subcommittee as it returns to work, or the finalizing of the plan could be delayed if SACATM wanted to provide more input. However, there will be no further drafts issued to the public for comment. Dr. Hansen noted that SACATM had already provided its input, and so would not see the impact without seeing another draft, which is apparently not planned. Dr. Wolfe said that any further input by SACATM would need to be done transparently in a public meeting forum. Dr. Stokes reiterated that the Implementation Plan would be the "living" document that would be updated periodically. He anticipated that a draft version would be publicly available after the January 2013 ICCVAM meeting, probably during the spring. He said that would be the appropriate time to provide feedback on the proposed activities. Dr. Hansen asked again whether the revised draft of the FYP would be made available to SACATM. Dr. Bucher said it could be made available, but any further input from SACATM would need to come in a publically convened teleconference, as Dr. Wolfe had mentioned.

Dr. Olson recommended that either the IWG be continued or a new working group constituted if SACATM desired to continue to develop the survey instrument established by the IWG.

Dr. Niemi asked how ideas for new working groups might be given to ICCVAM; whether they would need to be formulated during the current meeting, or if it would be acceptable to communicate after the meeting, and if so whether that would require a public forum. Dr. Bucher said it would be best to forward any recommendations during the current meeting, or after the meeting, recommendations could be forwarded to staff individually. Staff would consider the recommendations and communicate back to committee members as to the utility of any proposed working groups. He noted that there had been much discussion about the issue of communication between the agencies and the regulated communities and the idea of setting up public discussions, such as the ToxCast Communities of Practice model. He considered that as a good way for SACATM members to participate in ongoing dialogues, reporting back to the committee about their participation at the next meeting.

Dr. Hansen asked how the idea of sending agency heads letters requesting information for next year could be moved forward.

Dr. Niemi clarified that working groups are not created by SACATM, but must be established by NIEHS, although SACATM can propose working group topics. He added, "there appears to be significant interest in a working group approach to a pilot exploration of how best to gather animal use data from either regulators or regulatees, in partnership with those organizations and maybe trade organizations as well." He said that if such a group were formed, next year it would communicate back to SACATM what it has learned about an optimum process or form for a data capture spreadsheet on the numbers of animals used for which specific assays, under which specific pain or distress categories. Dr. Cavagnaro endorsed that approach.

XI. Project Concept Review: NICEATM Support Contract

A. Presentation

Supervisory Contract Specialist Melissa Gentry, NIEHS, briefed SACATM on the background of NIH project concept reviews and the criteria for the committee's review. Dr. Bucher cautioned that the discussion should be kept at a high level, without allusion to the specifics of the contract, so that no one present could gain any competitive advantage in bidding on the contract from having listened to the discussion.

As contracting officer representative, Dr. Stokes provided an overview of the project, including background information on the NICEATM support contract, with its history and composition. He delineated the activities involved in the support contract, which is designed to support and facilitate ICCVAM activities.

He described the proposed changes to the current statement of work: to include activities and expertise to support the goals stated in the draft NICEATM-ICCVAM 2013-2017 Five-Year Plan, and to provide expanded expertise in the emerging areas of bioinformatics and computational toxicology.

B. SACATM Discussion

Dr. Bunton, first discussant, noted the extent of NICEATM's duties and responsibilities. She said the contract is necessary, and felt that the updated activities are appropriate. She supported continuation of the activities using a contract mechanism.

Dr. Wilson, second discussant, said the operational support provided to NICEATM by the current contractor has been exceptional and a model of operational excellence. He agreed with the proposed expanded areas. He said he would highly support the implementation and continued maintenance of the contract.

Dr. Ochoa expressed some discomfort that he was being asked to vote on a contract about which he knew very little. Dr. Bucher explained that SACATM was being asked to vote on whether it believes that it is appropriate to use a contract to obtain the support NICEATM needs in its operation, and whether the proposed activities are appropriate to support the needs of NICEATM.

Dr. Niemi called for a vote. Dr. Cavagnaro moved to support using a contract mechanism for this activity. Dr. Bunton seconded the motion. SACATM voted unanimously (11 yes, 0 no, 0 abstentions) in favor of the motion.

XII. Updates on International Collaborations

A. Korean Center for the Validation of Alternative Methods (KoCVAM)

KoCVAM Vice Director Dr. Ki Hwan Choi updated SACATM on developments at KoCVAM, one of the ICATM member agencies. He described recent research activities on alternative test methods, international collaborations, other activities, and future activities.

He reported on the status of validation studies of a skin sensitization test involving a non-radioactive LLNA using flow cytometry and an *in vitro* skin irritation test using a reconstructed human epidermis model developed in Korea. A Korean CRO is participating in a JaCVAM-led international validation study on the SIRC cytotoxicity test method for eye irritation. Another Korean CRO is participating in an international collaboration to develop a guideline on a fixed-concentration procedure for acute inhalation toxicity. Four Korean labs are also participating in an international collaborative study to develop and *in vitro* test method for acellular pertussis vaccine.

KoCVAM is currently participating in several other international collaborative activities, including a workshop organizing committee, validation management teams, and a peer review panel. He described several other current KoCVAM activities, including adoption of a test guideline for an alternative test method to determine ocular toxicity of cosmetic ingredients and several other *in vitro* test methods for cosmetics.

Dr. Choi discussed the 4th KoCVAM Workshop for Experimental Training, held in May 2012, as well as other workshops and symposia in which KoCVAM was involved. Looking to the future, he said that KoCVAM anticipates adoption of further LLNA skin sensitization tests. KoCVAM will be in attendance at the 2013 International Congress of Toxicology in Seoul, South Korea. A workshop on alternative test methods and international regulatory perspectives will be scheduled at that event.

B. Health Canada

Dr. Tim Singer, Director of the Health Canada Environmental Health Science and Research Bureau, briefed SACATM on recent developments at Health Canada related to alternative methods. Dr. Singer provided background information on Health Canada, its research agenda, and noted the fact that it is an ICATM member. Although there is no Canadian center for alternative test method validation, Health Canada remains committed to sharing its expertise and collaborating to develop and implement non-animal alternatives for regulatory testing.

Dr. Singer said Health Canada is involved with developing alternative safety testing strategies for acellular pertussis vaccines, pre-validation study of cell transformation assays, detection methods for botulinum neurotoxin activity, and development of a chemical method of detection of paralytic shellfish toxins, which he described in more detail as an example of the agency's contributions to reductions in animal use (an annual saving of more than 40,000 animals). He noted that Health Canada is actively involved with the OECD Working Party of National Coordinators of the Test Guidelines Programme, and continues to provide expertise to various ICATM initiatives, particularly in pre-validation research. He cited the example of his organization's development of *in vitro* versions of the MutaTM-Mouse transgenic rodent mutation assay for hazard identification of chemicals, by which cell lines are derived from the mutant mice and are used to predict adverse effects of exposures.

Dr. Singer said that going forward, Health Canada will continue to develop, promote, investigate and/or implement alternative toxicity methods where they have scientific expertise. The agency will also continue its active involvement in the OECD Test Guidelines program, and will continue its engagement as an ICATM partner.

C. Japanese Center for the Validation of Alternative Methods (JaCVAM)

Dr. Hajime Kojima, Secretary General of JaCVAM, updated SACATM on recent activities at JaCVAM, an ICVAM partner. JaCVAM was established in 2005, and was modified last year to reflect added responsibilities involving the application and approval for manufacture and sale of pharmaceutical products, as well as revisions to standards for cosmetic products.

Dr. Kojima outlined the Japanese system for a new or revised test method, which begins with the JaCVAM Steering Committee, which he described as being similar to ICCVAM. Since JaCVAM was re-organized in 2011, the Steering Committee has an advisory council that plays a similar role to SACATM, and there is a body called the Regulatory Acceptance Board that approves new test methods.

Dr. Kojima described JaCVAM's organization and workflow in more detail. He delineated the methods accepted by the Regulatory Acceptance Board in 2011 and 2012, and the methods currently under consideration. He also described international and domestic collaborative efforts, as mandated by the 2011 re-organization. They include collaborations in validation studies, peer review panels, and other cooperative initiatives, including contribution to OECD Test Guidelines. He provided a history of a JaCVAM validation effort, the *in vivo* Comet assay. He said the main goal of JaCVAM is to ensure that new or revised tests are validated through comparison with domestically developed or internationally certified standard tests, peer reviewed, and officially accepted by the regulatory agencies. Under the ICATM framework, JaCVAM expects to experience more efficient test validation and review, as well as more rapid international acceptance of scientifically valid methods.

D. European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM)

Dr. Patric Amcoff of the European Union Reference Laboratory for Alternatives To Animal Testing (EURL ECVAM) presented *A Snapshot of Ongoing Activities at the EURL ECVAM*, which is one of the ICATM member partners. He noted that the organization's name had changed recently with the addition of the "EURL."

Dr. Amcoff said the mandate for the organization is contained in a new European Union (EU) Animal Welfare Directive issued in 2010 (DIR 2010/63). Annex VII of the directive delineates the full duties and tasks of the Union Reference Laboratory, which Dr. Amcoff described in detail. EURL ECVAM is situated within the European Commission's Joint Research Center (JRC), Institute for Health and Consumer Protection (IHCP), under the scientific units concerned with validation of alternative methods and systems toxicology. These two units will merge to be called systems toxicology, as of October 1, 2012.

Dr. Amcoff discussed what was new in EURL ECVAM since the 2010 Directive, noting that member states had two years to implement its provisions. Several advisory structures and processes are due to be set up and functional by early 2013. He described the procedures for engaging external experts to comprise the ECVAM Expert Pool (EEP), including paying them for their services. He also described the ECVAM Scientific Advisory Committee (ESAC) and the ECVAM Stakeholder Forum (ESTAF), as well as other networks such as the Preliminary Assessment of Regulatory Relevance (PARERE), ICATM, and OECD. He delineated the workflow involved with validation of alternative methods by the different groups, with the OECD being the ultimate customer to be reached by EURL ECVAM test methods with an aim toward inclusion and international regulatory acceptance as OECD Test Guidelines. He related the ongoing work being done by EURL ECVAM, which includes 9 validation studies and 14 test methods, in the areas of endocrine disruptors, genotoxicity/carcinogenicity, eye irritation, ecotoxicity, skin sensitization, and metabolism/toxicokinetics. He also described a new network established under the 2010 Directive, the Network of Laboratories for the Validation of Alternative Methods (NETVAL). It is a network of nominated laboratories invited to participate in or coordinate validation studies – currently there are 38 nominated laboratories from 12 EU member states. The hope, he said, is to use NETVAL to speed up the validation process considerably.

He described four ongoing ESAC Peer Reviews, such as the zebrafish embryo acute toxicity test (ZFET), as well as three others anticipated in 2013. He also reported on the status of several new OECD projects, which anticipate upcoming work by the EURL ECVAM. One of the group's strategies is to target endpoints of special regulatory concern – he cited skin

sensitization as an example of potential full replacement, and provided details about EURL ECVAM's strategic approach, including the use of the Adverse Outcome Pathway (AOP) concept as adopted by the OECD. He related the short-, medium- and long-term goals of the strategy.

Dr. Amcoff also briefly reported on a consortium called SEURAT-1, which is a research initiative involving nearly 100 scientists from over 70 European organizations. It is designed as a cluster of seven projects: five complementary research projects, a central data management and servicing project, and a coordination and support action. EURL ECVAM is a member of four of the groups and the consortium's steering committee. The culmination is expected to be the availability of several new alternative assays in 2016, at which time a SEURAT-2 initiative would commence

E. SACATM Discussion

Dr. Wilson asked whether U.S. scientists would be allowed to participate in the EEP, and if the ZFET would be intended to predict acute oral mammalian toxicology, or more an ecotoxicological endpoint. He encouraged ECVAM to work with the Registration, Evaluation, Authorisation, and Restriction of Chemical substances (REACH) authorities to formalize procedures to understand what would or would not be allowed for "read-across."

Dr. Amcoff said American scientists would definitely have the opportunity to register for the EEP database. He said the ZFET is intended to replace the acute fish test, OECD Guideline 203. He added there was considerable ongoing dialogue taking place between the European Chemicals Agency (ECHA) and JRC regarding read-across and other potential animal saving approaches.

Dr. Casey asked how many people work for EURL ECVAM, to help delineate some of the major differences between it and its U.S. counterpart. Dr. Amcoff said that EURL ECVAM has approximately 50 full-time employees, but there is a "loose capacity" of around 70 people as dictated by demand. Dr. Casey mentioned also that EURL ECVAM has its own laboratory with HTS capability. Dr. Amcoff confirmed that, and briefly described several of the EURL ECVAM laboratories, including a GLP compliant facility.

Dr. Stokes noted that EURL ECVAM and NICEATM-ICCVAM work in much the same areas, and work together to share experiences, avoid duplication of effort, and leverage resources to move projects along effectively. He said cooperation can significantly speed up adoption of alternative test methods at the OECD level, where it has been possible to get some adopted within less than 12 months. Dr. Amcoff agreed that harmonization of recommendations should be a prime objective of cooperation under ICATM.

XIII. Other Business

Dr. Stokes thanked the international colleagues for their presentations and their ongoing participation in activities related to alternative methods. He also thanked all of the participants in the meeting for their contributions. Dr. Bucher added his thanks as well, and expressed his appreciation for the many useful thoughts and suggestions. Drs. White and Niemi also thanked everyone.

Dr. Hansen asked about the request for letters to be sent to agency heads. Dr. Niemi said his impression was that that would be folded into the possibility of a SACATM working group, but it could be voted on at the meeting. Dr. Hansen called for a vote, recapping Dr. Elmore's comment requesting that SACATM send a letter to each agency head asking that they provide

data to SACATM next year on data relevant to animals use. He also asked that each agency with relevant data be given 10minutes to present at the meeting next year.

Dr. Hansen moved to send letters to agency head requesting data on animal usage. Dr. Chapdelaine seconded the motion. Dr. Ochoa said he was hesitant to support the motion, saying he felt unclear about what kind of data were going to be requested. He preferred folding the idea into a working group, who could flesh out the idea further before proceeding. Dr. Evans agreed, saying he would prefer to vote on a proposed draft letter, because otherwise it is a very open-ended request.

Dr. Bucher noted that NTP would likely look favorably upon a recommendation to form a new SACATM working group to pursue the matter. Dr. Ochoa said that there should be a motion to form such a working group.

Dr. Niemi called for a vote on the original motion, calling for the issuance of letters to agency heads. The vote was 1 yes, 9 no, and 1 abstention. Thus, the motion was defeated.

Dr. Hansen moved to give agency ICCVAM representatives time at the next SACATM meeting to report on the animal use data they had compiled and presented. Dr. Toth seconded the motion. Dr. Olson proposed the motion include broader discussion of acceptance of alternative methods by the representatives. Other SACATM members agreed to the modified motion. SACATM members voted unanimously (11 yes, 0 no, 0 abstentions) to accept the motion.

It was determined that it was not necessary to have a formal motion on the creation of a new SACATM working group, and Dr. Bucher said the idea would go forward.

Dr. Niemi adjourned the meeting at 2:45 PM, September 6, 2012.