

**Summary Minutes  
SACATM Meeting  
June 16-17, 2011  
Arlington Hilton, Arlington, VA**

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## **I. Location of Background Materials/Presentations and Frequently Used Abbreviations**

Background materials and presentations for the SACATM meeting are available on the SACATM meeting website (<http://ntp.niehs.nih.gov/go/7441>).

3Rs	Replacement, reduction, and refinement (causing less pain and distress) in the use of animals for toxicological testing
ACD	allergic contact dermatitis
BoNT	botulinum neurotoxin
BrdU	bromodeoxyuridine
BCOP	Bovine Corneal Opacity and Permeability
CDER	Center for Drug Evaluation and Research
CERI	Chemical Evaluation and Research Institute, Japan
CFR	Code of Federal Regulation
CM	Cytosensor Microphysiometer <sup>®</sup>
CPSC	Consumer Product Safety Commission
DACLAM	Diplomate, American College of Laboratory Animal Medicine
DA	Daicel Adenosine Triphosphate
DOD	Department of Defense
DPRA	Direct Peptide Reactivity Assay
ECVAM	European Centre for the Validation of Alternative Methods
EDC	endocrine disrupting chemical
EDSP	Endocrine Disruptor Screening Program
ELISA	enzyme-linked immunosorbent assay
EPA	U. S. Environmental Protection Agency
ER	estrogen receptor
EU	European Union
FDA	U.S. Food and Drug Administration
FYP	NICEATM-ICCVAM Five-Year Plan
GHS	Globally Harmonized System
h-CLAT	Human Cell Line Activation Test
HHS	Health and Human Services
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
JaCVAM	Japanese Center for the Validation of Alternative Methods
KoCVAM	Korean Center for the Validation of Alternative Methods
KFDA	Korean Food and Drug Administration

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LLNA	Local Lymph Node Assay
LTA	lipoteichoic acid
MAT	monocyte activation test
LPS	lipopolysaccharide
MUSST	Myeloid U937 Skin Sensitization Test
NCTR	National Center for Toxicological Research
NICEATM	NTP Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NIH	National Institutes of Health
NRC	National Research Council
NTP	National Toxicology Program
OECD	Organisation for Economic Cooperation and Development
PCRM	Physicians Committee for Responsible Medicine
rLLNA	reduced LLNA
ROS	reactive oxygen species
SACATM	Scientific Advisory Committee on Alternative Toxicological Methods
SBIR	Small Business Innovative Research
SOT	Society of Toxicology
STTA	Stably Transfected Human Estrogen Receptor Transcriptional Activation
TA	transcriptional activation
USDA	U.S. Department of Agriculture

## II. Attendance

SACATM met on June 16 – 17, 2011, at the Hilton Arlington, 950 North Stafford Street, Arlington, Virginia. The following individuals attended the meeting:

### **SACATM**

Laura Andrews, PhD, DABT, Genzyme Corporation  
Karen Brown, PhD, Pair O'Docs Enterprises  
Joy Cavagnaro, PhD, DABT, RAC, ATS, RAPS, AccessBIO, L.C.  
George Corcoran, PhD, ATS, Wayne State University  
Eugene Elmore, PhD, University of California, Irvine  
Steven R. Hansen, DVM, MS, MBA, DABT, ABVT, American Society for the Prevention of Cruelty to Animals  
Gwendolyn McCormick, DVM, MS, DACLAM, Boehringer Ingelheim  
Sharon A. Meyer, PhD, University of Louisiana at Monroe  
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  
Gary Wnorowski, MBA, LAT, Eurofins/Product Safety Laboratories

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**Liaison Representatives**

Michael Inskip, Health Canada (by telephone)  
Hajime Kojima, PhD, JaCVAM  
Sharon Munn, PhD, ECVAM (by telephone)  
Soojung Sohn, PhD, KoCVAM

**ICCVAM Primary Representatives**

Surender Ahir, PhD, OSHA  
Suzanne Fitzpatrick, PhD, DABT, FDA  
Jack Fowle, III, PhD, DABT, EPA  
T. Kevin Howcroft, PhD, NCI  
Steve Hwang, PhD, DOT  
Jodie Kulpa-Eddy, DVM, USDA, ICCVAM Chair  
Joanna Matheson, PhD, CPSC, ICCVAM Vice-Chair  
Moiz Mumtaz, PhD, ATSDR  
Paul Nicolaysen, VMD, NIOSH  
RADM William Stokes, DVM, DACLAM, NIEHS, NICEATM Director  
Margaret Snyder, PhD, NIH/OD  
Bert Hakkinen, PhD, NLM (by telephone)

**Other ICCVAM Representatives**

Raj Chhabra, PhD, DABT, NIEHS  
Vasant Malshet, PhD, DABT, FDA  
Richard McFarland, MD, PhD, FDA/Center for Biologics Evaluation and Research

**Invited Speakers**

Thomas Hartung, MD, PhD, Center for Alternatives to Animal Testing, Johns Hopkins University  
Kathy Kopnisky, PhD, NIH/OD  
John Vandenberg, PhD, North Carolina State University (retired)  
Daniel Shaughnessy, PhD, NIEHS  
Ward Tucker, PhD, Biosentinel, Inc.  
Jill Merrill, PhD, FDA

**NIEHS/NIH Staff**

Linda Birnbaum, PhD, DABT, ATS, NIEHS/NTP Director  
John Bucher, PhD, NTP Associate Director  
Warren Casey, PhD, DABT, NICEATM Deputy Director  
Robbin Guy  
Debbie McCarley  
Mary Wolfe, PhD, NTP Deputy Director for Policy  
Lori White, PhD, PMP, Designated Federal Officer

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**Other Federal Agencies**

Stephanie Devany, PhD, NIH/OD

Robert Mello, PhD, FDA

**Image Associates**

John Maruca

**SRA International**

Drew McMillen

Nathan Mitchiner

**Breakthrough**

Ernie Hood

**ILS Staff (NICEATM support contractor)**

David Allen, PhD

Steven Morefield, MD

**Public**

Lida Anestidou, PhD, The National Academies

Nancy Beck, PhD, Physician Committee for Responsible Medicine

Karl Johnson, Action Healthcare

Barbara Kochanowski, Consumer Healthcare Products Association

David Maillett, Edwards Lifesciences

Shazia Malk

Mary McBride, Agilent Technologies

Daniel Merkel, Product Safety Laboratories

Michael Usey, Certichem, Inc.

Catherine Willett, PhD, People for the Ethical Treatment of Animals

F. Naomi Zeytin, PhD, Biosentinel, Inc.

**June 16, 2011**

**III. Welcome and Introductions**

SACATM chair Dr. Steven Niemi called the meeting to order at 8:30 AM. All in attendance introduced themselves. National Institute of Environmental Health Sciences (NIEHS) and National Toxicology Program (NTP) Director Dr. Linda Birnbaum welcomed everyone to the meeting on behalf of NIH, NIEHS, and the NTP. She thanked the members of SACATM for their dedicated service on the committee. She recognized the presence and contributions of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) agency representatives and NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) staff in attendance at the meeting. She particularly thanked ICCVAM chair Dr. Jodie Kulpa-Eddy from the U.S. Department of Agriculture (USDA), and

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ICCVAM vice-chair Dr. Joanna Matheson from the Consumer Product Safety Commission (CPSC) for their leadership. She welcomed the international partners in attendance, Dr. Hajime Kojima from Japanese Center for the Validation of Alternative Methods (JaCVAM) and Dr. Soojung Sohn from the Korean Center for the Validation of Alternative Methods (KoCVAM). She mentioned liaisons from Health Canada and the European Centre for the Validation of Alternative Methods (ECVAM) would be viewing the webcast and making presentations by telephone later in the meeting. She noted that in March the ICATM Memorandum of Cooperation agreement had been updated to include Korea as a full member.

Dr. Birnbaum highlighted the public health role played by ICCVAM and NICEATM, emphasizing the important role the groups play in “protecting, promoting and advancing the health and safety of our citizens.” She announced that later that morning the Health and Human Services (HHS) Secretary and the Surgeon General would unveil the first-ever National Prevention Strategy, which would include recognition of the need for a healthy environment. She noted ICCVAM’s key role in translating the research advances and new technologies emerging from NIEHS/NTP activities into scientifically valid safety testing methods for regulatory use, which are important public health/prevention tools. She said new test methods are expected not only to be more predictive, but also to be faster, cheaper, and to require fewer or no animals.

Dr. Birnbaum said ICCVAM and NICEATM have now contributed to the endorsement or adoption of 42 new alternative methods, 28 of which are *in vitro* methods; over half of the *in vitro* methods use human cells. There are now approved alternative methods for many different types of testing including five of the six most commonly conducted safety tests. Last year, she announced that Federal agencies had accepted ICCVAM recommendations for updated procedures for assessing allergic contact dermatitis (ACD) that could further reduce animal use by 50% compared to the original version. She also forwarded ICCVAM recommendations to Federal agencies that are the first to incorporate green technology—two new versions of the local lymph node assay (LLNA), which do not require the use of radioactive materials. She noted progress on the validation of *in vitro* methods being coordinated with ICATM partners.

During the past year she also forwarded ICCVAM recommendations to Federal agencies on alternative methods and approaches for eye safety testing. Agencies have accepted the recommendations for the routine use of analgesics, anesthetics, and humane end points whenever animals must be used for eye safety testing. This will eliminate nearly all discomfort for testing situations in which animals must be used. Federal agencies also accepted recommendations for a third *in vitro* test method for assessing eye hazards in chemicals and products, the Cytosensor Microphysiometer (CM).

Dr. Birnbaum congratulated and thanked ICCVAM and NICEATM for their many accomplishments during the past year.

She presented the retiring SACATM members with a certificate and letter of appreciation for their service: Dr. Karen Brown, Dr. George Corcoran, Dr. Sharon Meyer, and Mr. Gary Wnorowski.

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NTP Associate Director Dr. John Bucher welcomed everyone to the meeting, and thanked the SACATM members for the preparatory work they had done and the work they would do during the meeting. He thanked Dr. Niemi for chairing the meeting.

Dr. Kulpa-Eddy expressed her appreciation to the SACATM members for the considerable time and effort they put in to the meeting, and said she and the other ICCVAM members were looking forward to their comments and recommendations. Designated Federal Officer Dr. Lori White read the conflict of interest statement for SACATM.

### **IV. NICEATM-ICCVAM Update**

NICEATM Director and ICCVAM Executive Director Dr. William Stokes updated SACATM on recent ICCVAM and NICEATM activities and priorities. He thanked the SACATM members for their participation, as well as the other stakeholders present at the meeting and joining by webcast. He noted the contributions of the ICCVAM representatives from 15 different Federal agencies, along with the scientists from these agencies who participate in the eight currently active ICCVAM interagency working groups.

Dr. Stokes' report included brief references to several items on the agenda for individual presentations later in the meeting.

- **Endocrine Disruptor Chemical Screening Methods:** The international validation study coordinated by NICEATM on the LUMI-CELL<sup>®</sup> stably-transfected transcriptional activation assay, which uses human ovarian carcinoma cells, was completed in 2010, followed by an International Peer Review meeting in March 2011. International validation of the MCF-7 Cell Proliferation Assay from CertiChem, Inc. was completed in March 2011; data are currently being analyzed and a review is expected later in 2011. The work is being coordinated by the ICCVAM Interagency Endocrine Disruptor Working Group, with liaisons from the ECVAM, JaCVAM, and KoCVAM.
- **NICEATM-ICCVAM International Workshop on Vaccine Potency and Safety Testing:** The workshop was held September 14-16, 2010. Nearly 200 scientists from 13 countries attended the meeting, which was co-organized with Health Canada, ECVAM, and JaCVAM. It addressed both human and veterinary vaccines. Proceedings will be published in *Procedia in Vaccinology* later in 2011.
- **International Workshop on Alternative Methods for Rabies Vaccine Potency Testing:** This workshop, to be held at the USDA National Centers for Animal Health in Ames, Iowa, October 11-13, 2011, is currently being organized by NICEATM-ICCVAM with ICATM partners. It will address alternative methods for both human and veterinary rabies vaccines, with attendees to include international scientific experts, regulatory authorities, and industry representatives.
- **Allergic Contact Dermatitis (ACD) Safety Assessment Methods: ICCVAM Evaluations:** Evaluations of several alternative methods for ACD safety assessments have been completed, the evaluation reports have been submitted, and the recommendations have been accepted and endorsed by both national and regulatory authorities. The evaluation report on the usefulness

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of the LLNA for potency categorization has been completed and has been forwarded to the Secretary HHS for transmittal to Federal agencies.

- ACD Safety Assessment Methods: Other ICATM Collaborations: ICCVAM and NICEATM are working with ICATM partners to validate several other *in vitro* and *in chemico* methods. ECVAM will also be conducting a peer review of KeratinoSens, a promising new ACD test method. In the fall of 2011, JaCVAM will begin a validation study of another *in vitro* skin sensitization assay that uses a human monocyte cell line.
- Ocular Safety Testing Methods: ICCVAM Evaluation and Recommendations: Evaluation reports have been completed and recommendations were forwarded to the agencies through the Secretary of HHS in September 2010. Recommendations were provided for ten different alternative test methods and strategies.
- Ocular Safety Testing Methods: Other International and ICATM Activities: A proposal to update the OECD ocular test guideline to include additional humane endpoints and routine use of analgesics and anesthetics has been submitted and was circulated to member countries for comments in June 2011. Adoption of a guidance document on the use of histopathology in ocular safety is expected by summer 2011. Several other methods are currently undergoing validation studies. Also, a replacement ocular battery (RoBatt) using existing *in vitro* test methods for eye injury assessment is being developed under the National Institutes of Health-Food and Drug Administration (NIH-FDA) Regulatory Science Grant program.
- Ocular Safety Testing: Using Fewer Animals to Identify Chemical Eye Hazards: Based on a request from CPSC, ICCVAM developed proposed criteria for hazard classification using a 3-animal test that would provide equivalent hazard classification as the current requirements that use 6 to 18 animals. The results of an analysis of 481 eye safety tests indicate that a criterion of 1 or more positive animals in a 3 animal test would provide the same or greater level of eye hazard labeling as current requirements. The new criterion will allow the 3-animal test to be used, which will reduce animal use by 50-83% compared to the current requirements. ICCVAM recommendations are currently in progress.
- Acute Systemic Toxicity Activities: NICEATM-ICCVAM is collaborating with ECVAM to develop *in vitro* models for human hepatic metabolism and toxicity. An acute dermal up-and-down procedure is also under development. The 3T3 neutral red uptake cytotoxicity test is under evaluation by ECVAM to determine if it can be used to classify "non-toxic" substances in the European Union (EU) without animal testing.
- Genetic Toxicity Test Method Activities: JaCVAM is leading international validation studies for *in vivo* and *in vitro* Comet assays. JaCVAM and ECVAM have made considerable progress on four types of cell transformation assays. The ICCVAM Interagency Genetic Toxicity Working Group and ICATM liaison have contributed to these efforts.
- Recent Test Method Nominations: New nominations include an *in vitro* pyrogen test method for assessing non-endotoxin pyrogens, and *in vitro* assays to detect and quantify botulinum neurotoxins.

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- **Developing Future Test Methods:** High throughput *in vitro* screening is taking place in the Tox21 collaboration, using the NIH Chemical Genomics Center's new robotic facility to screen 10,000 chemicals in an effort to identify toxicity pathways. NICEATM nominated over 900 chemicals for inclusion in the screening initiative, and recently nominated a nuclear receptor assay to use in the screening effort. The EPA's ToxCast™ program is also using 600 *in vitro* assays on a smaller subset of chemicals. NICEATM-ICCVAM will monitor the results of those studies for *in vitro* test methods with pathway-based predictive biomarkers.
- **Outreach Activities:** Recent NICEATM-ICCVAM outreach activities have included two workshops on best practices for regulatory safety testing, numerous posters, and an informational session on ICATM at the 2011 Society of Toxicology meeting. NICEATM-ICCVAM will have 11 presentations at the Eighth World Congress on Alternatives and Animal Use in the Life Sciences in Montreal in August.

Dr. Stokes concluded the presentation with a brief update on ICATM including the modification signed March 8, 2011 to add KoCVAM to ICATM.

## V. Regulatory Acceptance of ICCVAM-Recommended Alternative Test Methods

Dr. Stokes provided SACATM with an update on regulatory acceptance of ICCVAM-recommended alternative test methods, both in the U.S. and internationally.

- **LLNA for ACD:** ICCVAM recommendations for two non-radioactive LLNA versions and for an expanded applicability domain of the LLNA were transmitted to Federal agencies on June 12, 2010. He described several 3Rs-related advantages of the LLNA, which was first recommended by ICCVAM in 1999 as an alternative to the traditional guinea pig test method. In terms of reduction in animal use, the 2009-updated ICCVAM LLNA protocol uses 20 animals per test, versus a minimum of 30 in the guinea pig protocol and 25 in the original LLNA (a 20% reduction). Regarding refinement, the LLNA avoids the pain and distress associated with guinea pig tests since it does not involve the elicitation phase of ACD. ICCVAM previously recommended a reduced LLNA (rLLNA) for substances deemed unlikely to cause an ACD response, which uses just 12 animals per test, a 40% reduction compared to the standard assay. With regard to the two non-radioactive LLNA versions and the expanded applicability domain, agencies concurred with ICCVAM recommendations where applicable to their agency, although the FDA did note limitations of the LLNA- Daicel Adenosine Triphosphate (DA) assay, including a potential for false positives in the weakly positive response range. The new and updated LLNA-based test methods have been formally adopted and published by the OECD.

*Ocular safety testing:* Federal agencies recently indicated their acceptance of ICCVAM recommendations for the routine use of analgesics, topical anesthetics, and humane endpoints for required *in vivo* ocular safety testing. The OECD proposal for incorporation of these recommendations in the international guidelines is under consideration and is expected for adoption in 2012. ICCVAM has recommended the use of the CM *in vitro* method to identify substances not requiring ocular hazard labeling. ICCVAM reviewed the Bovine Corneal Opacity

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and Permeability (BCOP), Isolated Chicken Eye (ICE), Isolated Rabbit Eye (IRE) and Hen's Egg Test – Chorionallantoic Membrane (HET-CAM) tests to have insufficient predictivity for non-severe eye hazard categories, and has recommended additional optimization studies for each method. At EPA's request, ICCVAM evaluated an *in vitro* testing strategy to assess eye irritation potential for antimicrobial cleaning products. There were insufficient data to make a recommendation at this time, but EPA has initiated a pilot study to encourage further data submission. ICCVAM has recommended that use of the low volume eye test be discontinued. Federal agencies have agreed to ICCVAM's recommendations regarding ocular safety test methods, where applicable to their agencies.

Dr. Stokes noted the international acceptance of *in vitro* methods for acute systemic toxicity testing, OECD Guidance Document No. 129, published July 2010, that is based on the 2008 ICCVAM Evaluation Report that recommended the *in vitro* methods. The recommendations, which can reduce animal use by up to 50%, were also endorsed by Federal agencies in 2008.

For dermal irritation testing, OECD adopted a new test guideline in July 2010 for use of three reconstructed human epidermis test methods. For genetic toxicity testing, OECD formally adopted the *in vitro* micronucleus assay in July 2010.

### **SACATM Discussion**

Lead discussant Dr. Gwendolyn McCormick said it would take engaged, integrated teams to effectively implement any of the alternative methods systems. She recommended their availability be promoted to an even wider audience, to help implement the 3Rs, and that they be incorporated into training programs for laboratory personnel worldwide. SACATM and NICEATM-ICCVAM should focus more on test methods that help prevent disease and promote animal wellbeing. She recommended the establishment of awards programs in the 3Rs as incentives. Dr. Birnbaum said she concurred with the idea of increasing recognition for individuals, particularly getting professional organizations to do so.

Lead discussant Dr. Michael Olson said the wording of the first discussion question implied that some of the methods are not currently embraced, or that uptake within the regulatory community is very slow. He said he was not sure that is actually the case. As an occupational toxicologist, he has seen adoption of many of the alternative technologies, and those tests are in the mainstream of concerns on a daily basis. He recommended the establishment of a metric of acceptance and utilization within the regulatory community. Given that gaining acceptance and utilization is a long-term process, he recommended persistence and institutional commitment to the effort by NIEHS and other interested organizations. He considered the recent NICEATM-ICCVAM workshops great models for how the methods could be promoted, by bringing together regulators, the regulated community, and providers. He particularly endorsed the use of case study models at the workshops as an excellent tool for promotion, and recommended some form of publication of those materials. He noted there are some major geographic areas missing from the ICATM partnership—such as China. Regarding suggestions for ways to improve implementation and use of the alternative methods by the regulated community, he again endorsed workshops and recommended looking for opportunities to reach

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the regulated community by incorporating learning in venues where the community members would already be in attendance. He said there is a shortage of concise dictionaries or libraries of available alternative techniques. He suggested expanding the library currently available at the ICCVAM website or at another domain.

Lead discussant Dr. Daniel Wilson concurred with previous comments and suggested solicitation of stakeholder feedback early in the development process of new methods, to help understand practical, real world constraints on implementation. He said regulatory drivers are particularly important to understand. From an industry perspective, international regulatory acceptance would be important to implementing a new method. Outreach should be expanded to include emerging markets such as China and India. He said end users often have a good idea of what the outcomes of tests will be for a given compound, and use that information to streamline the testing process, so embracing early feedback from end users would further implementation of methods once they are approved.

Lead discussant Mr. Wnorowski asked Dr. Stokes about the adoption of the non-radiolabeled LLNA, which he understood EPA has not adopted. Dr. Stokes said when ICCVAM forwards recommendations to the agencies through the HHS Secretary, or by Dr. Birnbaum as her designee, the agencies have 180 days to respond by letter to ICCVAM. At that time agencies can either accept the methods or provide reasons why they are not accepting them. He said that constitutes a short period of time for response, and to actually modify agencies' formal guidelines may take longer. He added that in his presentation he was referring to the EPA's acceptance response by letter regarding its agreement with the recommendations. He noted the mutual acceptance of data (MAD) agreement with OECD requires U.S. agencies to consider data generated in accordance with OECD adopted test guidelines; however, agencies may require additional data. He asked ICCVAM EPA representative Dr. Jack Fowle to provide additional clarification.

Dr. Fowle said Dr. Stokes had explained the situation accurately; EPA does accept methods that are approved by ICCVAM, including the non-radiolabeled LLNA. The rLLNA has been accepted and has gone through the EPA's Science Policy Council for updating its guidelines, and that that process is ongoing. The policy has been sent to laboratories and registrants and there is an approved statement of language, but it is not on EPA's website yet. A public announcement should be on the EPA website soon. Registrants can provide data to EPA, ask questions, and EPA may request additional information to make sure the data meet regulatory requirements. Mr. Wnorowski noted the EPA policy document regarding the rLLNA had specifically mentioned that it did not constitute acceptance of the non-radiolabeled assays, and that they would not be considered until full adoption by the OECD with the publication of new guidelines. He asked specifically about the adoption of the non-radiolabeled test, pointing out some discrepancy among documents he had read.

Dr. Fowle said the EPA would accept those data on a case-by-case basis, and would evaluate the information with respect to what is needed to inform agency decisions. He said although a registrant might make a compelling case for use of the method, EPA would have to err on the side of public health protection and safety, and so would need to be very careful in evaluating

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methods both in the international and domestic contexts. He said there are systems in place to do so, but perhaps the process does not move as quickly as some would like. He said the EPA wants to ensure it is protecting public health, with a careful, transparent, systematic process that involves input from all stakeholders.

Dr. Steven Hansen asked if there was a tracking mechanism in place to count the number of submissions that reach agencies using the alternative methods, i.e., a simple barometer to assess whether animal use is actually being reduced. Dr. Stokes said that comment and question have come up repeatedly through the history of SACATM. He said that kind of data is not required to be collected or turned in by facilities that use animals. He said the one agency that does collect that type of data is EPA. Dr. Fowle said EPA had recently reviewed the submission of alternative methods versus traditional methods and he agreed to provide that information later during the meeting.

Dr. Linda Toth wondered whether the assays are actually better in terms of sensitivity, or just use fewer animals, and asked about the cost of the methods. She also expressed concern about the possibility of false negatives with the tests. Regarding the issue of adoption, she wondered whether the problem is with the regulatory agencies or with the regulated entities. Dr. Stokes said in terms of cost, which was evaluated several years ago, it appeared that the costs associated with the alternative methods were comparable to those of the traditional methods, at least for the LLNA vs. the traditional guinea pig tests. Regarding the sensitivity issue, he noted that for ACD there were human data available for comparison, and that the guinea pig and the LLNA had the same predictivity for predicting human sensitizers. Further, in the most recent review of LLNA data, the LLNA predicted everything that the guinea pig assay predicted in terms of positives, and actually predicted positives that had been negatives in the guinea pig test. Dr. Toth said such advantages, for any *in vitro* test, should be emphasized. Regarding false negatives, Dr. Stokes said in most of the *in vitro* assays that are approved as screening tests, negatives must be confirmed by animal tests. He said there are some false negatives, and that it would be important to clarify which substances and which properties contribute to them. Dr. Birnbaum added that in testing tens of thousands of substances using the Tox21 approach, substances that “light up the boards” in hundreds of assays are clearly a problem, but compounds that are negative in dozens of assays appear to be clearly not problematic. She felt as data are compiled, particularly in compounds about which a great deal is already known in terms of *in vivo* effects, confidence in negative results from large numbers of *in vitro* assays will increase.

Dr. Joy Cavagnaro said the case-by-case approach necessary with new methods made it more difficult to justify them, but it must be done to ensure acceptance. Dr. Fowle agreed, saying the context of the ICCVAM recommendation must be taken into account. It's important to begin with the end in mind and to work very closely with the primary users, which in his opinion in the United States are the EPA and FDA. Dr. Fowle said there needs to be a focus that addresses the real major problems the various agencies face in terms of how the tests will be used and what stakeholders are involved. The focus of ICCVAM is currently on the science, but there are other dimensions that should be considered in the future.

Dr. Ricardo Ochoa agreed with the importance of gaining regulatory acceptance for the alternative tests in emerging countries. He felt great pains were taken to reduce the risk of false negatives during the process of validating new test methods, so that they would be within an acceptable range. Regarding the issue of carcinogenicity testing, he noted the 2-year mouse bioassay is no longer required in Europe, but is still the default in the United States where perhaps it should be eliminated. He said he was pleased the Ocular Toxicology Working Group is evaluating histopathology as a possible way to add sensitivity to the tests, which would make it easier to reduce the required number of animals.

Dr. Eugene Elmore commented about the screening mechanisms used in toxicology studies. He had visited the National Chemical Genomics Center (NCGC) the previous day, to learn about the Center's work and how it might apply to the work of SACATM. He felt, for example, that it might be useful to access adverse event data from the FDA once drugs are on the market. He added he would like to see some guidance from SACATM and ICCVAM to NCGC to aid them with how to package cell-based tests, with the ability to assess particular human targets.

## **VI. Report on Peer Review Panel Meeting: Evaluation of an *In Vitro* Estrogen Receptor (ER) Transcriptional Activation (TA) Test Method for Endocrine Disruptor Chemical (EDC) Screening**

*Introduction and Overview of Proposed Methods and Applications: The BG1Luc ER TA (LUMI-CELL<sup>®</sup>) Test Method to Identify Substances with Estrogen Agonist and/or Antagonist Activity*

NICEATM Deputy Director Dr. Warren Casey briefed SACATM on the proposed endocrine disruptor test method. The EPA has been mandated to develop a screening program to detect EDCs so it asked ICCVAM to evaluate existing validated *in vitro* EDC screening tests. ICCVAM found none, leading ICCVAM and SACATM to make validation of such a test a high priority. In response, there was a nomination from Xenobiotic Detection Systems (XDS) for its LUMI-CELL<sup>®</sup> assay, a luciferase reporter assay that detects estrogen-binding activity. The assay is based in human ovarian carcinoma (BG-1) cells, with endogenous ER-alpha and ER-beta. The test provides a concentration-response, and so can assess both potency and efficacy. There are nearly identical protocols for both agonists and antagonists. The agonist assay involves gain of function, while the antagonist test measures loss of function, both based upon luciferase levels.

Dr. Casey provided a timeline for the project, beginning in January 2004 with the nomination of the assay by XDS, through the public peer review meeting in Bethesda in March 2011. He reviewed the definition of validation and ICCVAM's validation criteria, as well as the four phases of the international validation study, which was sponsored by NICEATM-ICCVAM, JaCVAM, and ECVAM.

When the testing was completed, accuracy and reproducibility were assessed. The agonist test method was 97% accurate, had 96% sensitivity and 100% specificity. The antagonist method was 100% accurate, with 100% sensitivity and 100% specificity. The agonist method showed 100% intra-laboratory reproducibility of the substances tested independently three times. Inter-

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laboratory reproducibility was 81%. For the antagonist methods, intra-laboratory reproducibility was 100%, while inter-laboratory reproducibility was 89%. In a comparison of the BG1Luc ER TA with the ER binding assay, there was 97% concordance. Compared with the Chemical Evaluation and Research Institute (CERI) Stably Transfected Human Estrogen Receptor Transcriptional Activation (STTA) assay, overall there was 86% concordance using 26 reference substances. Based on the validation program, ICCVAM recommended the use of the BG1Luc ER TA as a screening test to identify substances with estrogen agonist and antagonist activity, with the highest test substance concentration limited to 10  $\mu$ M for the antagonist assay. ICCVAM also developed and released performance standards for the assays.

ICCVAM conducted a peer review panel meeting March 29-30, 2011, to consider the recommendations, performance standards, and background data. The panel consisted of 16 scientists from 6 countries. Following the SACATM meeting, the Endocrine Disruptor Working Group will consider SACATM comments and the panel report and finalize ICCVAM's test method evaluation report. Ultimately, in fall 2011, the ICCVAM recommendations will be forwarded to Federal agencies, and a draft test guideline will be forwarded to OECD.

### *Summary of the Independent Scientific Peer Review Panel Evaluation of the Validation Status of the LUMI-CELL ER® (BG1Luc ER TA) Test Method*

Dr. John Vandenberg of North Carolina State University (retired), who chaired the Peer Review Panel ("the Panel"), briefed SACATM on the meeting.

He reviewed ICCVAM's charges to the Panel and its recommendations. The Panel agreed with ICCVAM that the BG1Luc ER TA could be used as a screening tool to identify substances with *in vitro* estrogen agonist and antagonist activity. It considered the test method protocol to be complete and adequate in detail, and agreed with ICCVAM about the needs for future studies. The Panel also suggested that such future studies could address metabolic activation, that the reference substance list and associated database could be expanded with additional negative agonist and positive antagonist substances as they are identified, and that efforts could be made to identify a quantitative cytotoxic method. It also concurred with the draft ICCVAM performance standards and some modifications to expand applicability of the performance standard.

### **Public Comments**

Dr. Niemi called for public comments and noted written comments had been submitted from CertiChem, Inc.

Dr. Catherine Willett, Associate Director of Regulatory Testing for People for the Ethical Treatment of Animals (PETA), reported that PETA lauded the Panel and supported the recommendations, both the main finding recommending the test method and the other recommendations. She congratulated the Panel on its review, saying it was "an incredibly thorough, well-done, well-reviewed validation study." She listed several panel recommendations that PETA supported: (1) designation of the assay as an alternative for the CERI STTA assay and the rat uterine cytosol assay, (2) development and validation of ER

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binding assays using recombinant receptors for both humans and other animals, (3) development and use of a metabolism component, (4) inclusion of potency evaluations to quantify activity, (5) evaluation of the quality of the data used to classify the original ICCVAM reference substances (6) discussion of the use of assay, and (7) discussion of the animal reduction potential.

She conveyed several additional PETA recommendations: (1) revise the chemical list to follow up on the evaluation and updating of the chemical reference list, and adding the new information to a publicly searchable database; (2) ensure that the best characterized chemicals are used for future assay evaluations; (3) identify new reference chemicals in underrepresented chemical classes; (4) consider the use of the assay to reduce animal testing, such as its use in addition to screening and prioritization, revising the structure of the Endocrine Disruptor Screening Program (EDSP) Tier 1 assessment by performing *in vitro* assays prior to animal testing, and the adoption of a weight-of-evidence approach that could be used to further reduce or eliminate estrogen receptor-related animal tests; and (5) evaluate the data quantitatively using a Relative Potency Index relative to a standard reference chemical, to allow quantitative comparison to the CERI STTA and to other assays

She noted the study had taken 7 years to complete, and so was not included in Phase I of the EDSP. She said a more efficient process is needed in light of the large number of new assays emerging. She recommended the Panel note issues that contributed to the length of the review in its report, and include recommendations for avoiding those issues in future reviews.

Dr. Niemi recognized Dr. Fowle, who was at that point prepared to respond to Dr. Hansen's request regarding data on adoption of alternative test methods.

Dr. Fowle said data had last been collected August 26, 2010, regarding 12 assays, which were grouped from the larger assay population: LLNA: 241, Corrositex: 0, Up and Down Assay: 1,139, EpiSkin/EpiDerm: 2, BCOP: 14, ICE: 0, *In Vitro* pyrogen tests: 0, Cytosensor: 0, EpiOcular: 3, LumiCell: 0, CertiChem: 0, Total: 1,399.

He mentioned that those figures may make it appear that EPA and others are not committed to reducing, refining and replacing animal use, and asked that he be allowed to comment at some point about some of the things EPA is doing to achieve the 3Rs. Dr. Niemi asked Dr. Fowle to hold those comments for later in the meeting.

### **SACATM Discussion**

Dr. Corcoran, lead discussant, said the EDC method evaluation seemed to be "a tour de force," and commended the work of the Panel. He said he would like more information about the quality of data issue that had been commented upon in the Panel's report, specifically the criteria involving ranking and sensitivity analysis, or tests for trends in terms of the criteria for evaluating positive and negative compounds. He asked Dr. Vandenberg to comment on whether the Panel was proposing a higher and new standard for all assays of this nature. Dr. Vandenberg said it would be presumptuous for the Panel to do so, in terms of attempting to direct what other panels might do. On the other hand, he said, it would be fine for other panels

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to adopt the standards described by this one. Dr. Corcoran asked for clarification on the Panel's conclusion that there were insufficient data to term the evaluation a "thorough" analysis, although it was termed as "adequate." This was, he said, due to the use of a descriptive versus a formal, inferential assessment of the data. Dr. Vandenberg said it was hard for the Panel to consider the analysis to be thorough, since there would always be things that had not been thought of. Thus, their description of the analysis was adequate. Statistically, he said the analysis of the data was considered to be adequate, with no fault found. Dr. Casey added it was always difficult to get statisticians to agree on anything, so some of the comments pointed to ways things could have been done differently, statistically, particularly EC<sub>50</sub> calculations. Dr. Corcoran said he had been hoping to hear that ICCVAM was moving toward a new standard for quality of data.

Dr. Corcoran added he would like to have seen more information in the document on the implications of the assay for use in Europe and Japan. Dr. Vandenberg said that was not specifically discussed as it related to the background document, but it did come up during the discussion, and there were foreign representatives present who brought some of those issues. Dr. Corcoran said he would like to have seen validation conducted in one set of known agonists and antagonists, and then movement into a second set of yet-untested agonists and antagonists, thus incorporating a two-step process. He recognized it had already been a 7-year, \$3 million process, but nonetheless objected to validation based on only one set of compounds. Dr. Casey said every positive and every negative they could find had been tested, but the chemical space was very small for well-referenced compounds; just 38 compounds fit the criteria. Dr. Corcoran maintained since the protocol was changed over the course of the 7 years, having two sets of data would have helped, even if it involved splitting up the known compounds. Despite his comments, Dr. Corcoran said the review was "a very impressive body of work."

Dr. Elmore, lead discussant, agreed with the previous comments, as well as the conclusions and recommendations contained in the report. He felt, however, the BG1 cell line needs to be better characterized. He recommended the cell line be placed in a repository to ensure access and availability in the future.

Dr. Meyer, lead discussant, was also impressed with the work of the Panel, calling it "very comprehensive and very clear." She strongly supported the idea that cytotoxic changes be quantified. She noted that although validation normally means the replacement of an *in vivo* method with an *in vitro* method, in this case, an *in vitro* method is to be replaced by another *in vitro* method. She questioned the priority of whether ICCVAM should be funding such an effort, given the large number of animals still being used in other areas. Dr. Meyer noted the introduction of the non-radioactive LLNAs would actually replace animal use, but that the EDC assay is a screening method, and that she was uncomfortable with expending too many resources on such an approach. She wondered whether the current method could not be further developed to work on antagonists. She also asked about harmonization for *in vitro* methods. She mentioned it would be helpful to have a formula in the document on how the fold-reduction was calculated and commented on a lack of clarity for expressing the performance standard.

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Regarding priority, Dr. Stokes said the developer nominated the method for validation studies in 2005, and at that time it was given a very high priority by SACATM. He said in the case of a positive, that such information could be used along with other mechanistic data to move forward with characterizing whether or not the compound is in fact an *in vivo* endocrine disruptor. Dr. Stokes said regarding the comparison with the current method that has been adopted by the EPA and is in their guidelines, this was done because the adoption had occurred after the validation study was initiated. He said, "But we didn't even know about that method in 2005, that it even existed, but it was moving along and a couple of years later, yes, we did find out that it was going through validation as well. This study was nominated and a validation study was initiated before there was any knowledge of the other method."

Dr. Fowle said in terms of maximizing the utility of tests, clearly things have evolved, and some of the earlier screens that were developed for validation occurred a number of years ago. He said it's really important, if these screens get used, that they get linked very closely in terms of working with the regulatory agencies and the users who'll be using them, to make sure these assays will be used, and will be used for purposes which will help advance the mission. He said Dr. Meyer raised some very good points in terms of the resources available. ICCVAM focuses on validation of alternative methods to animal tests, and he thinks it's very important to focus on replacements for animal tests. EPA's policy and approach for using the EDSP is such that it probably will not be using this assay. He said he thought it just sort of underlines the importance of having very close communications at the beginning, middle, and end. He alluded to the history of EPA discussions with Drs. Stokes, Bucher, and Birnbaum as they tried to build on the lessons learned to try to do a better job in the future. He suggested having a retreat or similar meeting to look at the good things ICCVAM has done, see what might be improved, and figure out how to move forward. Dr. Birnbaum agreed with Dr. Fowle, but reminded everyone that the purpose of some *in vitro* tests is to answer a very specific question. She said this test determines whether a substance is an agonist or antagonist for ER $\alpha$  and ER $\beta$ , but there are other ways that chemicals can be endocrine disruptors, e.g., of the estrogen signaling system, and this test is not identifying them.

Dr. Wilson, lead discussant, concurred with previous comments, as well as the need for a follow-up meeting with ICCVAM to focus on trying to determine an overview of the various assays currently in use. He noted to run an assay is as much an art as a science, and that it should be moved more toward the science. So a focused discussion with experts to understand the limitations of the current assays and see whether any stand out would be helpful to further the state of the science. For the EDC assay, he agreed with Dr. Elmore regarding better characterization of the cell line. He cautioned that use of the phrase "endocrine disruptor" carries an obvious stigma, and suggested a careful definition of what is or is not an endocrine disruptor be put into the background information of the document.

Dr. Casey noted the figures regarding accuracy, reliability, and reproducibility had been approached in a thoughtful manner, with choices having been made among potential approaches. Dr. Meyer suggested revising the specific section she had earlier referred to as problematic. Regarding usability of the assay in high throughput screening, Dr. Casey said it was currently being evaluated at NCGC, and that it works well in a 384-well format, but it may

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not provide adequate signal-to-noise in a 1536-well format. He continued by noting the cells for the assay are co-owned by XDS and Dr. Michael Denison at the University of California, Davis, and that Dr. Denison was reluctant to put the cells into a repository because he wishes to maintain control of them. He does make them freely available to academic and government labs through a formal licensing process. Dr. Vandenberg said the Panel had discussed the issue of cell line availability at length, and did all they could to ensure access to the cell line. Dr. Toth expressed concern about drift in the cell line over time, asking whether there are quality control measures to ensure such drift would not take place. Dr. Casey said positive and negative controls are run with each test, but that currently there is not a way to track the genetic stability of the line. Dr. Stokes said all of the *in vitro* assays use acceptance criteria for the positive controls, so there must be a response within that acceptance range. Thus, if the cells have changed and the response has been decreased to below that threshold for an acceptable positive control response, or if it exceeds the upper limit of it, it would not be a good run and it would indicate that perhaps the cells had changed, become contaminated, or were the wrong cells.

Regarding the history of the assay, Dr. Stokes noted the EDSP was mandated by laws in 1996. The *LUMI-CELL ER*<sup>®</sup> was developed in response to a Small Business Innovative Research (SBIR) topic issued by NIEHS in the late 1990s in response to considerable interest at the time. The SBIR grant to develop the EDC method was supported by NIEHS and NIH grant funds. Dr. Birnbaum added that since NIH supported the development of cell lines, they should be fully available. Relevant to agencies' involvement, Dr. Stokes said there is an Endocrine Disruptor Working Group that includes representatives from all of the ICCVAM agencies. Dr. Stokes said the working group had EPA representatives on it who were kept abreast of the study design, chemical selection, and protocols, which were all run by that group before this testing went forward. He clarified that all of the agencies in ICCVAM had the opportunity for input into this validation study. New members have been integrated into ICCVAM and SACATM, and the work of the previous members may have been forgotten. He said NICEATM-ICCVAM is trying to make sure as much information as possible is reflected in the final evaluation reports that go out to the agencies and to the public.

## **VII. Federal Agency Research, Development, Translation, and Validation Activities Relevant to the NICEATM-ICCVAM Five-Year Plan: NIH Update**

NIH ICCVAM representative Dr. Margaret Snyder provided SACATM with an overview of NIH activities related to NICEATM-ICCVAM priorities not otherwise represented at the meeting. She passed the podium to Dr. David Allen, who directed the committee members to an online compilation of agency activities relevant to the NICEATM-ICCVAM Five-Year Plan (<http://iccvam.niehs.nih.gov/docs/5YRImplement/AgencyActivities2011.pdf>). Dr. Snyder said the NIH part of the table had been compiled from responses to a questionnaire she had sent out asking for listing of agency activities related to advancing the 3Rs. She noted that about 31 of the report's 39 pages described NIH agency activities. She said the activity to be presented at this meeting would be the NIH effort incorporated in the RFA *Advancing Regulatory Science*

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*through Novel Research and Science-Based Technologies.* The awards were announced in September 2010, and NIH is continuing its efforts with FDA.

### *NIH Regulatory Science Initiatives*

Dr. Kathy Kopnisky, NIH Office of Science Policy, described NIH initiatives involving regulatory science. On February 24, 2010, NIH Director Dr. Francis Collins, FDA Commissioner Dr. Margaret Hamburg, and HHS Secretary Kathleen Sebelius jointly announced the NIH-FDA Regulatory Science Initiative, which is intended “to accelerate the process from scientific discovery to the availability of new, innovative medical therapies for patients.” The plan involves interrelated efforts focusing on translational science and regulatory science. The goals expressed by the NIH-FDA Joint Leadership Council were to improve translational research, in part by making the agencies’ science “regulatory review ready,” and by incorporating the latest science into the regulatory review system. The RFA provides three years of funding, totaling approximately \$9 million, supported by the NIH Common Fund and the FDA. The RFA’s research goals are to develop new and improved:

- Biomarkers, models, and methods to predict safety and efficacy of regulated products
- Clinical trial design methodologies for evaluating safety and efficacy of medical products
- Bioinformatic tools to improve medical product safety and to consistently predict and assess complex drug interactions
- Methods for post-market detection and analysis of adverse events

Four cooperative research grants were awarded, addressing innovative approaches to clinical trial design, nanoparticle characterization, a novel strategy to predict ocular irritancy, and a heart-lung micromachine model to test the efficacy and safety of drugs.

Dr. Kopnisky described the Joint Leadership Council in more detail. It is co-chaired by Drs. Collins and Hamburg, with 12 additional individuals at the Institute or Center Director level, as members. Two meetings have been held to date, with two more scheduled for this year. The Council adopted criteria for the selection of projects, as well as a list of more than 50 potential collaborative projects, which were organized into six working groups according to thematic areas, including preclinical research, clinical research and trials, drug rescue and re-purposing, bioinformatics, statistical design and analysis, tobacco science, and “toward a shared culture.” Each working group will identify and pursue various sub-topics, including several related to toxicology and predictive toxicology. The hope is this new formalized interagency activity, along with other collaborative activities, will lead to improvements that will bring new therapies to patients faster and more efficiently.

### *SBIR/STTR Programs at NIEHS*

Dr. Daniel Shaughnessy, NIEHS Program Administrator, briefed SACATM on the SBIR and the Small Business Technology Transfer (STTR) programs that have been supported by NIEHS. In FY 2010, NIH SBIR grants totaled \$616 million and STTR grants totaled \$74 million. The institutes and agencies involved are required to set aside 2.5% of their budgets for SBIR, and

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0.3% for STTR. At NIEHS, that translates to approximately \$11 million per year for SBIR grants, and approximately \$1.3 million for STTR grants.

Dr. Shaughnessy described the three phases of the SBIR/STTR programs, which ultimately are intended to lead to commercialization, and the differences between the programs. At NIEHS, the emphasis in the programs is on improved test systems for prioritization and safety, tools for improved exposure assessment, technologies for measuring internal dose of environmental agents, and hazardous substances detection and remediation. He briefly described several examples of current grants, including two of the several 3D tissue culture projects being funded: a human corneal model for an ocular irritation assay, and a reconstructed skin micronucleus genotoxicity assay.

In addition to grants, NIEHS has solicited some targeted contracts over the years, several of which have supported NICEATM-ICCVAM and Tox21 goals. Dr. Shaughnessy described several current examples, as well as the current topics being solicited for contracts. He also illustrated several resources for more information on current programs and solicitations.

### **SACATM Discussion**

Dr. Corcoran, lead discussant, felt NIH was among the ICCVAM agencies with more capability than the others to contribute to the development of improved alternative safety methods. He said there is currently more of an opportunity for NIH to take center stage in regulatory and safety sciences than it has in the past. Support for toxicology at NIH had declined in recent years, particularly due to the recent reorganization of the study sections. He said current toxicology proposals to NIH “have very few homes,” with very few toxicologists on study sections. Due to the reorganized system, support for toxicology generically and for regulatory sciences and alternative testing has been impaired. Additionally, there has been no change in priority for safety assessment or alternative testing.

He felt the NIH and FDA “are to be applauded” for the Regulatory Science Initiative, but noted that it is a very small program, with limited capability to impact safety sciences or alternative tests. The program lacks the scope to change the landscape or the progress of regulatory sciences, or specifically, alternative safety testing. Much additional funding is used for Tox21 and computational methods/data analysis. He said the initiative seems to be a “boutique program” designed to show activity rather than to substantially move the field forward.

Dr. Birnbaum responded, stating the Center for Scientific Review had made efforts to address the problem with study sections, but there were still “orphans,” which may include alternative testing methods. She pointed out the NICEATM program costs NIEHS approximately \$3.4 million per year, and the NIEHS component of Tox21 costs roughly \$4-5 million per year, along with many grants in the area. She mentioned the \$15 million spent by NIEHS and NTP on nanoparticle safety and characterization. She concluded by stating that there are many opportunities to have some input into this regulatory science initiative and agenda, and NIEHS has a 20-year or more history working with the regulatory agencies to advance regulatory science. Dr. Corcoran agreed that NIEHS was a leader in this area.

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Dr. Hansen, lead discussant, expressed a desire to make sure that the priority list for the Regulatory Science Initiative is developed with a long-term vision. He was pleased to hear that EPA is now counting usage of alternative methods, and asked that next year there be a presentation that would include those numbers in comparison to historic numbers, including data from FDA and other users. That would allow SACATM to assess progress and perhaps drive resources into bottleneck areas. Dr. Niemi added that next year there should be information about the denominator – how many compounds or tests were conducted in total, versus the *in vitro* alternatives, to give an idea of whether the ratio is changing as well as the absolute numbers.

Dr. Olson, lead discussant, said he took a “rate and rank” approach to evaluate the contributions to the development of alternative testing methods, he felt the SBIR program was most important, followed by the NIH-FDA Regulatory Science Program, followed by general NIH research. He said NIH research generally did not have the development of new alternative test methods as its focus. He recommended the NIH-FDA program use targeted solicitations to elicit the appropriate expertise. He added that the use of targeting is what makes the SBIR program so viable. He recommended dialog between Regulatory Science Program grantees and NICEATM-ICCVAM staff during the development phase of research, allowing the opportunity for NICEATM-ICCVAM to “hear and steer” the progress of the research. He also recommended there be a mechanism for some further consideration for those who do not make the NIH-FDA payline, with meritorious ideas and proposals being re-routed to other funding sources such as SBIR. Resources, including test batteries and test chemicals, should be made available so grantees should not be allowed to work in a vacuum.

Dr. Wilson, lead discussant, noted the growth in predictive toxicology in recent years. One reason is the increased amount of and access to data in the field, and he hoped training and interactivity in large databases would continue to increase. He felt interoperability among databases would allow more *in silico* screening, more targeted *in vitro* screening, and perhaps decreased *in vivo* screening. Systematic approaches should be used to do modeling with data already in existence. He also urged the inclusion of metabolic capabilities based on tissue types in screening procedures, including Tox21 assays. He recommended more emphasis in Tox21 on whole-genome expression assays and on annotating assays initially. Dr. Bucher described the BioPlanet program in Tox21, which lays out 11,000 human biochemical and physiological pathways in graphic style. Tox21 assays can be overlaid on the graphic globe, and thus a picture of the coverage provided by Tox21 emerges, allowing the targeting of new assays to fill gaps. Regarding the idea of ICCVAM supporting *in silico* research, he said ICCVAM is evaluating but not presently actively supporting it.

Dr. Kohnsky said applicants who applied but were not successful in receiving awards in response to the Regulatory Science Initiative (RFA 10-006) did benefit from a written review from a study section, and were free to pursue the other normal sources of investigator-initiated funding. She said it would be useful for the awardees to have the opportunity to present their work to ICCVAM. She added that although the initiative seems small, it could be thought of as akin to a pilot, with much scientific expertise and support coming from the agencies to the investigators.

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Dr. Niemi said the new partnership between NIH and FDA is very wise, raising the bar on patient safety by leveraging the discovery assets at NIH, leading to new science faster, eventually leading to improved alternatives. He recommended a pharmaceutical consortium element be added, with appropriate protection of confidentiality, to expand access to patient samples, especially as personalized medicine enters the mainstream. He asked about the measurement of return on investment for SBIR programs. Dr. Shaughnessy said NIH had recently started a tracking system called the Performance Outcomes Data System (PODS), designed to measure the type of data suggested by Dr. Niemi.

Dr. Meyer expressed support for the R01 mechanism, noting that much of the SBIR program had emerged from research originally done within R01s. She cautioned about the notion of mining old databases, because the metrics from the older databases may not be directly applicable to current needs.

Dr. Toth said she would like to see more emphasis on translational and applied science in NIH training programs, rather than just basic science. Dr. Birnbaum said NIEHS supports 42 different training programs, many of which contain some focus on alternative methods development.

Dr. Fitzpatrick, FDA Office of the Chief Scientist in the Office of the Commissioner, said her office has the science lead on the Advancing Regulatory Science program. She noted the FDA is not a large grant-giving agency like NIH or EPA, but the unique part of the program's grants is that FDA scientists work hand-in-hand with the researchers, in recognition that for the new methods to be rapidly and efficiently incorporated into the regulatory framework, FDA needs to be talking to the developers early in the process, so that they can be steered toward developing the types of tests needed to answer the pertinent regulatory questions. She said even though the grants may not seem large in the realm of NIH, the researchers are being nurtured so their products will be able to go into FDA's regulatory framework. Dr. Snyder agreed, noting NIH is beginning to identify grants that have the potential to offer a new paradigm for regulatory science. She said the question is how the research is incorporated into alternative methods.

Dr. Stokes said it was clear that increasing communication between all of the stakeholders involved, from the R01 basic scientists to the companies taking that science and trying to apply it in new testing methods, is important, particularly communication with the regulatory agencies. He noted that facilitating that communication is one of the reasons ICCVAM was established. Integrating information from a variety of sources would be a new frontier for the development of methods, but that there needed to be enough certainty to make informed decisions, so ICCVAM must use "smarter strategies" to be able to make those decisions earlier in the process with good certainty. He said determining what additional tests would aid that process is vital to accomplishing that goal. Dr. Wilson responded that much of the emphasis for ICCVAM validation has been on the acute toxicity endpoints, but there are no acute toxicity data the ToxRef database with which to do modeling.

## **VIII. Nominations to ICCVAM**

### *In Vitro Pyrogen Assay Validation*

Dr. Richard McFarland, FDA and the ICCVAM Pyrogenicity Working Group chair, reviewed the process for nominations to NICEATM and ICCVAM. The process ranges from initial nomination of a proposed test method by a sponsor through NICEATM and ICCVAM preliminary evaluations, to a public meeting of SACATM, to final ICCVAM recommendations on priority and activities. He mentioned ICCVAM's five criteria for prioritization, most notably, applicability to regulatory testing needs and agency programs, and potential to reduce, refine and replace animal use compared to current accepted methods.

He introduced the nomination of an *in vitro* pyrogen test method for assessing non-endotoxin pyrogens. First, he reviewed pyrogen testing, as well as the five *in vitro* pyrogen test methods previously evaluated by ICCVAM, all of which were *in vitro* tests based on primary human blood cells or a human cell line. In 2007, the methods were reviewed at a NICEATM-ICCVAM Peer Review Panel meeting. In 2008, ICCVAM's final recommendations on the *in vitro* pyrogen tests were released. In that report, the limitations of the tests were delineated, including the fact that the scientific basis of the tests suggested they might be able to detect non-endotoxin pyrogens, but there were insufficient data to support broader application at that time. Future studies suggested in the report included endotoxin-spiked and non-endotoxin-spiked samples. In 2009 the ICCVAM recommendations for the five *in vitro* pyrogen tests were endorsed by the Federal agencies that regulate pyrogenicity testing, with the methods to be considered on a case-by-case basis for the detection of Gram-negative endotoxin in parenteral drugs, subject to product-specific validation

In 2011, ICCVAM received a nomination from Biotest AG for a monocyte activation test (MAT), that was previously reviewed by ICCVAM, for coordination of an independent validation study to evaluate the MAT for its ability to detect non-endotoxin pyrogens, as called for in the 2009 ICCVAM recommendations. Dr. McFarland described how the proposed test method would apply to the ICCVAM prioritization criteria he had reviewed, including its applicability to several regulatory testing needs. The test has a substantial potential to contribute to the 3Rs, since rabbit pyrogen tests use more than 300,000 rabbits per year. He said the nominated activity should be a high priority, and that further discussion should proceed to determine what additional information is needed to adequately characterize the usefulness and limitations of the MAT for identifying non-endotoxin pyrogens, including an assessment of data gaps and the studies needed to fill them, and identification of studies considered necessary to characterize the method's validation status for regulatory testing purposes.

### *Nomination of the Whole Blood Pyrogen Test for Extension to Non-endotoxin Pyrogens*

Dr. Thomas Hartung, Johns Hopkins University and the University of Konstanz, reviewed the scientific details of the proposed test. He said the 2009 acceptance of the endotoxin pyrogen test by the FDA and the European Pharmacopoeia was exciting, but there had been little use of the test due to the fact that it was not approved for non-endotoxin pyrogens. Thus, with the high degree of animal use involved (about ten times as many rabbits as are used for eye and skin

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irritation testing), it remains a priority activity. He noted the *Limulus* test, aside from potentially endangering horseshoe crabs, does not reflect the potency of pyrogens in humans.

The proposed test uses cryopreserved whole human blood cells, with little need for processing; a distinct advantage over other methods. He related details and data from the original validation studies. The validated methods using cryopreserved cells were found to be an important standardization that would be widely applicable due to increased availability of primary cells, the ability to exclude abnormal donors, and the ability to exclude infectious agents. The tests are reproducible and robust and perform well in terms of predictive capacity, which should augment regulatory acceptance.

Dr. Hartung said the challenges include the fact that there is not a single well-characterized non-endotoxin pyrogen that can be used as a reference. But the issue of patient safety makes the need to detect non-endotoxin pyrogens clear, especially with the advent of new expression systems in gene technology, and new medical devices, cellular therapies, and the emergence of environmental pyrogens. He also pointed out that the ICCVAM recommendations for future studies from 2008 would have cost approximately \$20 million to fulfill precisely and completely, but unlike the previous validation studies, in this case there is no public money available to validate the proposed assays. Thus, the proposed study is "lighter" than the recommendations, but still attempts to fulfill the essential requirements involved. He said the desire is to be involved with ICCVAM at an early stage, because it will require a major investment to validate the study, despite the fact that by the time it is approved, patent protection will have expired.

Dr. Hartung said the main problem is how to address the non-endotoxin bacterial pyrogens. He favors the idea that lipoteichoic acid (LTA) is the principle endotoxin of gram-positive bacteria. Thus, LTA is proposed as the non-endotoxin standard in gram-positive bacteria for the MAT validation study. Dr. Hartung presented data comparing LTA with lipopolysaccharide (LPS), which is known to be the principle endotoxin in gram-negative bacteria. Although his group does endorse the use of LTA, they recognize that there are potential problems with the approach, including LTA instability, difficulty of achieving a presentation effect for potency, differences between the mouse and the human (LTA is more potent in humans), the fact that it is not a complete endotoxin, its limited commercial availability, and the fact that there is no specific test comparable to the *Limulus* assay. Thus, Biotest AG seeks advice from SACATM to help address those problems and design a validation study intended to meet ICCVAM's criteria. They invite ICCVAM to be directly involved with the validation management group they are setting up. They seek ICCVAM involvement with the choice of test materials and non-endotoxin pyrogens. Biotest AG wishes to work in the "spirit" of GLP. They suggest using LTA and some lysates of gram-positive bacteria. They feel a parallel rabbit test is not feasible, nor is it ethical, consuming hundreds or perhaps thousands of rabbits, but would use rabbit tests sparingly, to demonstrate whether the spike is pyrogenic. There are potential savings in reproducibility as well, since the assay has been validated previously in many labs.

### **Public Comment**

Dr. Niemi noted a written comment from PETA and the Physicians Committee for Responsible Medicine (PCRM). Representing PETA and PCRM, Dr. Nancy Beck said the groups support expanded approval of the MAT test that has been nominated, believing it has the potential to be more accurate and a more sensitive assay than those currently in use. She provided their suggestions for the expanded validation plans.

First, she went over some of the scientific background on pyrogens, and the immune response to pyrogens, including a summary of interleukin-1 $\beta$  induction by pyrogens via toll-like receptor signaling, a process shared by both endotoxin and non-endotoxin pyrogens. Thus, there is substantial literature to support the expanded use of the MAT, she said, and it should have been approved for biologics and devices when it was first validated in 2008. She noted her groups recommend the expanded validation of MAT be accomplished by (1) reconsideration of parallel testing for a prospective validation study; (2) reconsideration whether a large-scale prospective validation study is even necessary, particularly given the fact that companies may have to perform product-specific validation studies regardless of ICCVAM validation; and (3) consideration of coordination of smaller, product-specific validation studies.

Dr. Beck shared several other more specific suggestions: (1) expanding efforts to collect data, (2) holding a best practices workshop, (3) using metrics to assess pyrogenicity testing, and (4) performing an updated literature review.

### **SACATM Discussion**

Dr. Brown, lead discussant, noted she was chair of the original peer review panel for pyrogenicity testing. She noted the existence now of a test kit with cryopreserved blood from pre-screened donors answers many of the questions that arose in the original peer review. After describing her long experience with LTA assays, she said she strongly agreed that this is a high priority for evaluation and that rabbit testing should be replaced with other tests to detect non-gram-negative pyrogens. She suggested rather than running a full, very expensive validation study, ICCVAM could work to incentivize companies that are currently running the rabbit assays, or that have products needing this type of testing, to run the proposed assays alongside the rabbit testing. That would potentially accomplish validation less expensively and perhaps more appropriately, with relevant products. Confidentiality would of course be essential, she added.

Dr. Cavagnaro, lead discussant, agreed the proposed assay has high applicability to regulatory testing needs. She felt the proposal for LTA was supported, but asked Dr. Hartung to elaborate about the "limited supply" of the material. He said his laboratory no longer has the capacity to produce LTA, but there are still 400mg left available for validation purposes. There is also a commercial source, he added. Dr. Cavagnaro asked Dr. Hartung whether his group had looked at any other types of cell therapies for the assay. He said they had looked at three others, but not in any controlled trials.

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Dr. Meyer, lead discussant, asked Dr. Hartung to elaborate about the scope of the problem, and what percentage of testing would detect a non-endotoxin pyrogen. He said that was a difficult question, because the historical choice to conduct pyrogen testing in rabbits tended toward gram-negative endotoxins. He added that only by having the proposed assay available and using it on non-endotoxin pyrogens would the extent of the problem be learned. Dr. Meyer asked whether a considerable number of products were getting through that were non-endotoxin pyrogens. He said the Good Manufacturing Practices had brought down the incidence considerably; however, there is tremendous underreporting of fever reactions from the clinic, when they are found to be associated with intravenous drugs. She asked about costs associated with the study, since he had asked for help identifying potential savings. He said the rabbit testing is definitely the most expensive element, so if a great deal of rabbit testing was required, the study would quickly reach non-feasibility. He said the assay itself is not very expensive.

Dr. Meyer said the test being biologically feasible supports its adoption. She suggested ICCVAM put together some performance standards for *in vivo* tests used in validation if they are not very informative and complicate the validation.

Dr. Toth, lead discussant, noted there seemed to be problems with the rabbit assay and it is, thus, not very robust. She wondered whether the regulatory agencies require pyrogenicity testing, or testing for the presence of a pyrogen. If pyrogenicity is what is important, she said, the rabbit test might provide more public health security with its ability to detect the range of physiological reactions. She also asked how characterized and standardized the source of blood for the assay is, and whether it is possible to screen for non-responders. Dr. Hartung explained the cryopreserved blood is typically pooled from at least five donors, leveling out potential abnormalities. He said in hundreds of samples, they had not seen non-reactors or over-reactors to the pyrogens. He added that although the *Limulus* test had replaced approximately 90% of pyrogen testing, the remaining 10% was still conducted in rabbits due to some problems with the *Limulus* test, due to its inability to detect non-endotoxin pyrogens. Thus, he felt the peer review panel had put forth an unrealistic requirement for the non-endotoxin pyrogen test in looking for every non-endotoxin pyrogen to be shown.

Dr. Brown agreed with Dr. Hartung that the bar had been set extraordinarily high for non-endotoxin pyrogens. She asked Dr. McFarland whether the FDA is getting feedback about products with non-endotoxin, non-gram-negative pyrogens. Dr. McFarland said he could not comment specifically about matters under investigation, but that it is a topic frequently discussed in the development of the biotechnological products, and is an issue dealt with on a daily basis. She asked if he thought the companies involved might be willing to run the proposed assay in parallel with the rabbit test. Dr. McFarland said he could not speak for industry, but he would be willing to suggest the idea. Dr. Cavagnaro felt that without a viable alternative, the companies might well be willing to test the assay. Dr. Hartung summarized the use of the assay by many companies up to this point, including device manufacturers, since the assay has the advantage of being conducted in a liquid medium, and thus can be used to test surfaces. He added that many companies might not be willing to participate in validation studies, since they would not wish their products to be associated with contamination. Thus, he

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said, it would make sense to adopt the modular approach to validation as endorsed by OECD. Dr. Stokes said this was a case that called for flexibility on the part of ICCVAM. He felt the intent of the Pyrogenicity Working Group was to determine what needs to be known and how to get that information, and that what ICCVAM is requesting from SACATM is to advise whether the test is a priority for further action and discussion.

Dr. Hartung responded to Dr. Meyer that Biotest is proposing to identify three laboratories to run a variety of samples that will cover previously studied intravenous drugs, other therapies, and devices. Biotest would use LTA and gram-positive crude extract to spike the samples. Parallel test data would be collected in rabbits and with the *Limulus* assay. He said the cost would be determined by what the laboratories are willing to provide.

Dr. Niemi clarified that what SACATM was being asked to vote on was not the validation study itself, but to advise ICCVAM to move forward with flexibility to pursue rational validation activities. Dr. Ochoa mentioned that although SACATM should not be designing the study, he agreed that Dr. Hartung and his group should form a validation management group to advise on the design of the study. Dr. Niemi explained that SACATM was to vote on whether to advise ICCVAM to pursue the study with high priority and to consult with Biotest to design whatever follow-on activities might be necessary.

Dr. Cavagnaro moved to accept prioritization of the proposal. Dr. Toth seconded the motion. SACATM members voted unanimously in favor of the motion.

### **Nomination of *In Vitro* Assays for the Detection and Quantification of Botulinum Neurotoxins**

#### *Introduction*

Dr. Kulpa-Eddy provided background for the nomination of *in vitro* assays for the detection and quantification of botulinum neurotoxins (BoNTs). She said BoNT is the most toxic substance known, and it causes much sickness and death in the U.S. in humans and animals. The FDA approved BoNT in 1998 as a therapeutic agent, and by 2008 there were an estimated 8 million cosmetic treatments using BoNT in the United States. It is also a bioterrorism threat.

In 2006, NICEATM-ICCVAM and ECVAM sponsored a workshop on alternative methods for botulinum toxin testing. The workshop panel determined that none of the assays that had been reviewed were ready to be a complete replacement for current methods, and recommended further development and validation efforts for replacement alternatives. A nomination has been received from BioSentinel Pharmaceuticals for three *in vitro* assays: BoTest™, BoTest Matrix™, and BoCell™. They are nominated for consideration for interlaboratory validation studies to evaluate the extent to which they can (1) detect and quantify BoNT in a wide range of samples, (2) determine drug product potency, and (3) diagnose clinical botulism.

In terms of prioritization criteria, the ICCVAM Biologics Working Group determined that the assays would (1) be useful to several Federal agencies, including FDA and USDA; (2) reduce or replace significant animal use (one mouse potency bioassay can use up to 300 mice; BoNT

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therapeutic product regulation accounts for an estimated 600,000 animal deaths per year); (3) be applicable to complex samples; (4) offer the promise of providing high throughput applications; (5) not require extensive instrumentation or specialized training; and (6) offer significant cost savings over traditional animal-based methods.

Thus, ICCVAM proposed a high priority for further discussion to determine what is needed to adequately characterize the usefulness and limitations of the nominated assays.

Dr. Ward Tucker, Research Director of BioSentinel Pharmaceuticals, Inc., described the company's scientific and business goals, and provided an overview of the products: the BoTest™ consists of two products—BoTest™ A/E and BoTest™ B/D/F/G, BoTest™ Matrix is also two products—BoTest™ Matrix A and BoTest™ Matrix E, and BoCell™ is a cell-based assay. All three measure endopeptidase activity, but differ in their applicability. The BoTest™ products are mix-and-read, with no secondary reagents needed. The BoTest™ Matrix products are designed to detect and quantify BoNT in complex matrices such as serum, blood or environmental samples. It detects proteolytic activity following an immunoprecipitation step. The BoCell™ line detects BoNT proteolytic activity after the toxin exerts its other activities of cell receptor binding, internalization and translocation, and thus, is intended to provide a more complete picture of what the toxin does.

Dr. Tucker further described the mechanisms of action of the assays, and presented sample data from each of them.

The BoTest™ kits were commercially released in 2009 and 2010, and have been used in governmental and commercial laboratories. They have been used to screen more than 150,000 compounds to date. BoTest™ Matrix A was released in May 2011; BoTest™ Matrix E will be released in summer 2011. The matrix assays are compatible with field samples. The BoCell™ line will be released in summer 2011. It is an engineered and highly selected cell line, and will be offered as a licensed product only.

Dr. Tucker described the company's efforts related to manufacturing, quality assurance, and product validation, which are designed to provide consistent products that are tailored to client needs, with guaranteed results.

### **Public Comment**

Dr. Niemi noted a written comment from PETA and PCRM and recognized Dr. Beck, speaking for PETA and PCRM, who said that the organizations support the nomination.

### **SACATM Discussion**

Dr. Cavagnaro, lead discussant, asked Dr. Tucker whether the BoCell™ test was second-generation versus the BoTest™ assays, and anticipated it to be the assay of choice eventually. Dr. Tucker said the company had recently received an NIH SBIR grant to develop a second generation BoCell™ assay, to address current sensitivity issues, and anticipate it will be the assay of choice for many applications, especially in drug product manufacturing. However, an environmental sample could not be run on a cell-based assay, so the biochemical assays will

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still have many applications. With some validations in progress, Dr. Cavagnaro asked Dr. Tucker what the proposed validation/s would be. Dr. Tucker said the company was looking for guidance from SACATM and ICCVAM on that question. He agreed with Dr. Cavagnaro that the key validations would probably be on the BoTest™ Matrix and BoCell™ assays. Dr. Cavagnaro asked Dr. Tucker about potential competitors, and Dr. Tucker described two other companies and their products relative to BioSentinel's, noting the BoTest™ Matrix assays are the first activity-based assays on the market that can deal with complex matrices, and that there are no other cell-based assays on the market. Dr. Cavagnaro asked about the validations that have been conducted at various companies already, and whether those validations could be compiled together to aid the overall validation. Dr. Tucker said the pharmaceutical clients set their own validation parameters, and data sharing is up to the clients.

Dr. Hansen, lead discussant, said he supported the assessment of the data gaps and moving as quickly as possible to identify studies that need to be done to move forward with validation.

Dr. McCormick, lead discussant, concurred with Dr. Hansen.

Dr. Ochoa, lead discussant, said he supported moving forward with priority to conduct the required tests, but would like to see more of a focused approach to clarifying what kind of tiered use of the tests would be performed. Dr. Tucker said when they know what they are testing, protocols solidify themselves quickly. Dr. Ochoa wondered about how to know how long to incubate samples containing unknown quantities of the toxin. He also asked what happens with prolonged incubation—whether there is an increase in false positives. Dr. Tucker said there is, especially with “dirtier” samples, but the phenomenon can be controlled with reporters that have the cleavage sites knocked out.

Dr. Brown inquired about karyology being done on cells and how putrid samples are analyzed. Dr. Tucker said BioSentinel is starting karyology and is reaching out to other companies that are testing contaminated samples. Dr. Brown noted BioSentinel is already working with several companies doing their own validations, and wondered about the focus of an ICCVAM validation. Dr. Tucker said the focus would be more on environmental testing, rather than clinical testing.

Dr. Niemi called for a motion and vote. Dr. Ochoa moved the proposal be accepted. Dr. Brown seconded. The SACATM members voted unanimously to accept the proposal.

## **IX. Report from the ICCVAM Workshop Series on Best Practices for Regulatory Safety Testing**

### *Assessing the Potential for Chemically Induced Eye Injuries*

Dr. Jill Merrill, FDA and chair of the ICCVAM Ocular Toxicity Working Group, updated SACATM on the Best Practices Workshop for assessing the potential for chemically induced eye injuries held at NIH Bethesda on January 19, 2011. There were 77 attendees, mostly from government and industry, and there were 16 ocular posters. More than 90 people viewed the webcast of the workshop's plenary sessions online.

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Dr. Merrill summarized the pre-workshop communications efforts that had been conducted. The workshop's program included a discussion of public health impact, examination of currently available ocular test methods, U.S. requirements for consideration of alternatives, current guidelines for safety testing, a roundtable discussion with the relevant regulatory agencies, and consideration of case studies. Ocular safety test methods discussed included pain management and humane endpoints to always be considered when *in vivo* testing is still required, the BCOP, the ICE, and the CM test methods and their respective validation status.

New ocular safety test methods currently in the validation pipeline were also discussed. These included tests being considered in the ECVAM Eye Irritation Validation Study (EpiOcular™ and SkinEthic™), other non-animal test methods used alone or combined in a specific strategy for labeling Antimicrobial Cleaning Products, and the JaCVAM 2<sup>nd</sup> Validation Study (Short Time Exposure Test).

A post-workshop survey with 26 responses showed substantial satisfaction with the event. Dr. Merrill said several attendees told her that in future workshops they would like to see breakout sessions with more case studies demonstrating the effective use of non-animal test methods for assessing ocular hazard classification. The workshop participants were presented with four case studies, designed to illustrate the decision criteria based on the known usefulness and limitations of the ocular methods currently available.

### *Assessing the Potential for Chemically Induced ACD*

Dr. Joanna Matheson, CPSC and vice chair of ICCVAM, briefed SACATM on the January 20, 2011 Best Practices Workshop on assessing chemically induced ACD held at NIH Bethesda. There were 77 attendees, mostly from government and industry, and there were 18 ACD posters. More than 90 people viewed the webcast of the workshop's plenary sessions online.

Dr. Matheson summarized the pre-workshop communications efforts that had been conducted. The workshop's program included a discussion of public health impact, examination of currently available ACD test methods, U.S. requirements for consideration of alternatives, a roundtable discussion with the relevant regulatory agencies, and consideration of case studies. The ACD safety test methods discussed included the traditional LLNA, the rLLNA, the LLNA: BrdU-ELISA, the LLNA: DA, and the Direct Peptide Reactivity Assay (DPRA). New ACD safety test methods in the validation pipeline discussed included the DPRA, the Human Cell Line Activation Test (h-CLAT), and the Myeloid U937 Skin Sensitization Test (MUSST). While each method alone may not be able to generate sufficient information to make a hazard decision, when taken together as part of an integrated testing strategy, they could at a minimum be used for screening with the goal to potentially be seen as full replacement assays.

There were 19 respondents to the post-workshop survey, who indicated the event was well received. There were three case studies presented: one on the potential for reducing animal use by using the validated LLNA, and one each on how to conduct and interpret results from the validated nonradioactive LLNA methods, the LLNA: BrdU ELISA and the LLNA: DA.

### **SACATM Discussion**

Dr. Elmore, lead discussant, praised the organizers for the workshops, which he had attended and found very productive and informative. He appreciated the updates on the current test methods, and the opportunity to interact with regulators. He said he would like to see a system in place for gaining U.S. regulatory acceptance of new test methods once they are accepted by OECD. He suggested the regulatory agencies issue detailed explanations, in the form of user's guides, of what to submit—particularly elements they may expect to see but are not specified in guidelines. He said he had learned much from the regulatory roundtables at the meetings, and felt the breakout sessions were very informative. He said the LLNA was an example of the difference between official acceptance of a method and its use in actual practice. He discussed the EPA and other agencies regarding acceptance of such tests. He suggested putting a plan in place, once a test is proposed for validation, to address implementation. This could avoid extensive delays in providing information to both their regulators and their stakeholders in the industry. He said the sooner approved tests could be implemented, the better.

Dr. Olson, lead discussant, had also attended the workshops, had discussed them with Dr. Elmore, and concurred with Dr. Elmore's assessment. He felt in both meetings there should have been more time allocated to discussion among the scientists of sharing information on the appropriate use of results in regulatory safety testing, and to discussion of challenges of incorporating alternative test methods into regulatory safety testing guidelines. He would like to have had more attendees from regulatory agencies, particularly personnel directly involved with regulatory submission reviews, who could comment on particular areas of concern. He also suggested there be consideration of holding future workshops in a more central venue, despite the advantages offered by the Natcher Center.

Dr. Ochoa, lead discussant, said he did not have the opportunity to participate directly in the workshops, but was impressed with the materials that had been prepared for the meeting, especially the case studies. He felt generally that the issue of the challenges of incorporating alternative test methods into regulatory safety testing guidelines is very important and needs to be discussed more with the regulators. He attributed the resistance to fear of change, comfort with the older tests, fear of jeopardizing public health, and a lack of consciousness that the world is changing.

Mr. Wnorowski, lead discussant, agreed with previous comments that the breakout sessions and exchanges between government and industry representatives were very important. Those exchanges help to eliminate the misunderstandings regarding appropriate testing. He considered the workshops' attendance as good, and that it would be difficult to increase attendance by moving future sessions out of Washington, as that venue is so convenient for the regulatory community.

Dr. Cavagnaro discussed the need for global alignment of the international validation groups to further acceptance of new methods.

Dr. Meyer asked if there had been any update on the issue of false positives in the LLNA that had been discussed last year. Dr. Stokes said the issue had been discussed with FDA

representatives at the workshop. The FDA had found more positives with certain dermatologic products that didn't turn out to be positive during clinical trials. He added the intent is to have similar workshops every year or two on whatever methods are of the greatest interest. He appreciated the feedback he had received about what those future topics should be. Dr. McCormick noted that fewer than 50% of the workshop attendees had given feedback on the sessions, and suggested using anonymous electronic polling to get higher response rates and honest feedback. Dr. Stokes reminded attendees that all of the PowerPoint presentations from the workshops are available on the ICCVAM website, as is the entire webcast.

**June 17, 2011**

## **X. Report and Recommendations from the NICEATM-ICCVAM International Workshop on Vaccine Potency and Safety Testing: State of the Science and Future Directions**

### **A. Presentation**

Dr. Kulpa-Eddy briefed SACATM on the workshop, which was held September 14-16, 2010 at the Natcher Center at NIH in Bethesda. The workshop, which addressed both veterinary and human vaccines, was attended by nearly 200 scientists from 13 countries, and included presentations by regulatory authorities and experts from industry and academia.

Vaccine potency and safety testing had been identified as one of the highest priorities of NICEATM-ICCVAM in the five-year plan, due to the large number of animals used, the fact that the tests involve significant unrelieved pain and distress, and the multiple agencies involved. Thus, the goals of the workshop were to (1) review the state of the science of available 3Rs alternative methods for vaccine potency and safety test methods and discuss ways to promote their implementation; (2) identify knowledge and data gaps that need to be addressed to further advance alternative methods for vaccine potency and safety testing; and (3) identify and prioritize research, development, and validation efforts needed to address those gaps.

The workshop consisted of an opening plenary session, three sequential plenary sessions, and breakout groups following each of those sessions. Priority lists were generated in the breakout sessions.

In terms of veterinary vaccines, participants determined a list of criteria for prioritization of future 3Rs efforts, and recommended priorities for those efforts, segmented into priorities for potency testing and for safety testing. They assessed the state of the science for *in vitro* and serological potency assays, and suggested a series of priority activities for *in vitro* and serological veterinary vaccine potency assays. State of the science and priority activities were discussed with regard to veterinary vaccine potency assays with earlier humane endpoints. Priority activities were delineated for reduction alternatives for potency assays and for veterinary vaccine safety testing assays. The attendees compiled a list of recommendations for global progress in alternatives for veterinary vaccine testing.

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Dr. McFarland summarized the human vaccine activities at the workshop. He emphasized that the plenary sessions were all held in one room, with the veterinary and human vaccine researchers together to facilitate dissemination of knowledge between the two fields. They were separated in the breakout sessions, which is where the priority lists were generated. For the human vaccine potency and safety assays, criteria for prioritization of future 3Rs efforts were listed, as were recommended priorities. The state of the science and priority activities for *in vitro* and serological human vaccine potency assays, and for use of earlier humane endpoints, were described. Priority activities were also listed for reduction alternatives for human vaccine potency assays, and for human vaccine safety testing alternatives. Participants also described recommendations for global progress in alternatives for human vaccine testing.

Dr. McFarland alerted SACATM to a NICEATM-ICCVAM Workshop on Rabies Vaccine Potency Testing, which will be held October 11-13, 2011 at the USDA Center for Veterinary Biologics in Ames, Iowa.

### **SACATM Discussion**

Dr. Laura Andrews, lead discussant, suggested the information emerging from the workshop be disseminated to a wider audience. She said the companies that are developing vaccines did not seem to be significantly represented at the meeting. In terms of the first stage projects, she wondered about the criteria for success, so that the one final study would be very definitive, noting that in biopharma, that was the standard. She felt the priority lists presented by Drs. Kulpa-Eddy and McFarland were comprehensive and aggressive, but it might be more effective to pick one or two priorities from each list that are “truly achievable.” She wondered if it would be possible, once potency is established, to design an *in vitro* assay that would be predictive of what would be seen *in vivo*. She said guidance from a group such as SACATM could help address the issue of repeat testings *in vivo*. She suggested tapping a consortium of industry experts who might be willing to share their knowledge.

Dr. Brown, lead discussant, said she had attended the workshop and found it to be excellent. She felt there had been extremely good interaction between the regulators and the industry. She was particularly pleased with the interaction and participation on the animal health side, which she noted she had not seen in previous meetings. She noted the progress in the animal health industry toward using alternative methods had been quite slow over the years. She complimented NICEATM-ICCVAM on its contributions to moving the field forward, noting that there had been some major accomplishments in the last year. She said there had been much discussion at the workshop about how to handle so-called “legacy” products—how to convert those laboratory animal tests to *in vitro* assays without going through an unaffordable process. She reported that a draft memorandum dealing with that issue had emerged from the conference, particularly allowing industry to re-qualify *Leptospira* products with laboratory animal methods and get *in vitro* methods in place more quickly. She noted that the same memorandum contained a recommendation on new products coming through the system as of 2011—that industry interact with USDA at the inception of a project, which she deemed a very positive step. Regarding the upcoming rabies vaccine workshop, she said the products are much the same for both human and animal vaccines, and that the two areas should be able to

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work together to move alternative methods forward. She discussed the difficulty of designing *in vitro* assays in which the adjuvant does not interfere with the assay. She recommended that experts be brought into the workshop to discuss best practices for extraction of adjuvants and conduct of *in vitro* assays. She recommended that master/standard references be monitored to ensure they are remaining stable.

Dr. Cavagnaro, lead discussant, said she had attended the plenary sessions of the workshop. She recommended instituting a method for all to have access to successful implementation of alternative methods, suggesting that was something ICCVAM could make available through its website. She was also impressed with the interactions with regulatory agencies.

Dr. Toth, lead discussant, said she had not had the opportunity to attend the workshop. She noticed an apparent discrepancy among the discussants in their assessments of the level of industry participation at the meeting. She also wondered about the recommendations that emerged from the meeting, noting she had not seen them in the materials provided, and had not been exposed to them prior to the presentation at this meeting. Dr. Stokes said there were recommendations from each of the breakout sessions, and that they were being published as a separate paper, which would be completed within the next month or two. Dr. Toth hoped the timeline could be shortened in the future. She felt the determination of what is the best test would be more effective in encouraging adoption by regulators and the regulated community, emphasizing advantages such as cost-effectiveness and repeatability along with 3Rs considerations.

Dr. Wilson said there should be consideration of monitoring body temperature in test animals and refinement of the guinea pig vaccine test, in addition to the workshop's recommendations. Dr. Toth responded that rather than looking for refinements, validated alternative endpoints should be sought. Dr. Stokes said there had been considerable discussion of humane endpoints at the workshop, and it would be treated in the forthcoming publication. He felt that in fact industry had been well represented at the meeting. Total animal use for biologics testing is almost twice that of toxicity testing, along with a high degree of unrelieved pain and distress, so from a 3Rs standpoint, biologics testing is an important target for ICCVAM.

Dr. Brown said there might in the near term be reports of increased animal use as industry converts to *in vitro* tests, since they will need to be validated against the animal tests.

Dr. Niemi regretted he had missed the workshop. He felt assays that put humans at risk should be eliminated, and that the occupational safety element should be considered. In terms of refinement, he challenged industry and the regulators to be more aggressive and look for replacements, at least by looking at moribundity and illness as "less inhumane" endpoints than death, or even looking for alternatives that would not involve a live animal's experience at all. He noted at present most vaccines are preventive, but soon therapeutic vaccines would be coming on line, perhaps presenting an opportunity for the vaccine side of the pharmaceutical industry to be a model in finding better alternatives.

Dr. McFarland wished to note SACATM's role in the process of organizing the workshop. He said with prophylactic vaccines currently in development, both FDA and USDA seek to

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encourage industry not to default to animal tests for potency, but to go directly to *in vitro* tests from the outset if the science supports this. He noted FDA had approved the first therapeutic cancer vaccine last year, Provenge for prostate cancer, and its potency test is a completely *in vitro* test.

Dr. Niemi brought up the SACATM charter, which he said is due to expire in December. He suggested the wording of the charter might need some editing, particularly where it addresses SACATM assignments. He noted there is opportunity in the charter for the establishment of subcommittees, and felt there might be value in looking at a subcommittee charged with looking at actual implementation or adoption of alternative assays that have gone through the NICEATM-ICCVAM validation process, as well as international processes. He noted that at this meeting there had been comments from agency representatives that they may or may not use certain assays, despite the fact that they had been through the process, and said the subcommittee he was proposing may be a good way to investigate why that was so.

Dr. Olson expressed his full support for Dr. Niemi's suggestions regarding revisiting the charter and formation of a subcommittee. Dr. Hansen questioned how many ICCVAM members were participating in the meeting. He felt the ICCVAM representatives at the meeting should be presenting their activities to SACATM, rather than the reverse. He said in his third year of SACATM membership, he wondered if he had made any impact. Dr. Ochoa supported Dr. Niemi's subcommittee proposal.

Dr. Meyer asked whether industry must still conduct its own internal validation studies when ICCVAM has validated and endorsed a method. Dr. Stokes said that what had been heard the previous day addressed two different segments of testing, consumer products (regulated by CPSC), and pesticide products (regulated by EPA), where there is generally no need for product-specific validation, which is required by FDA. Dr. Brown said it is not generally possible for companies with many products to simply adopt a test method for all of its products. She had been proposing that agencies or institutions hold standard references that are global and could be used by all of industry, for instance in rabies or tetanus vaccines, where the human and animal products are basically the same.

Dr. Bucher commented on Dr. Hansen's comment about the effectiveness of SACATM. He said it was a typical problem with advisory boards consisting of people with short, overlapping terms; it is difficult to get the long view of the group's accomplishments. He said his impression is that SACATM is one of the most engaged advisory panels he has seen, with highly influential advice and directions. He encouraged members to not be discouraged, but to take the longer view and take great pride in the panel's achievements. Dr. Elmore said he had seen much progress by ICCVAM, but SACATM could and should do more to promote implementation of validated tests, and thus he supported Dr. Niemi's proposal.

Dr. Moiz Mumtaz, ICCVAM representative from CDC, supported Dr. Bucher's comments, and said he had been impressed by the energy displayed by SACATM. He said there are limits on funds for travel for ICCVAM members to attend meetings. Regarding the issue of acceptance of

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methods, he said just because a method is approved, its acceptance, and particularly integrating that in the government guidance procedures, takes a long time.

Dr. Stokes echoed Dr. Bucher's comments about SACATM's contributions. He said the committee's activities have evolved since it was established in 1997. He praised the SACATM's significant contributions to advancing the 3Rs and coming up with better, more scientifically advanced methods. Dr. Cavagnaro mentioned the importance of agency involvement throughout the process as new alternatives are developed, in order to help ensure acceptance and implementation.

Dr. Corcoran recommended that future SACATM meetings begin with a brief review of highlights of the actions that have resulted from SACATM members advising ICCVAM. That, he said, would be one way to gauge the impact of SACATM's work.

## **XI. Updates on International Collaborations**

### *Korean Center for the Validation of Alternative Methods*

Dr. Soojung Sohn, Vice Director of KoCVAM, updated SACATM on the center's activities. She showed an aerial photo of the Osong Health Technology Administration Complex, a campus where several Korean regulatory and scientific agencies, including the Korea Food and Drug Administration (KFDA), are located, along with several corporations. As of November 2010, the KFDA and the National Institute of Food and Drug Safety Evaluation, of which KoCVAM is a part, relocated to the Osong campus. Validation of laboratories and animal facilities at the site have been completed.

Dr. Sohn described the signing ceremony for the modified ICATM Memorandum of Cooperation, which took place March 8, 2011, in Washington, DC and which supported the membership of KoCVAM in ICATM.

She introduced KoCVAM's international collaborations, beginning with international validation studies. A KFDA laboratory took part in ICCVAM's lead *in vitro* endocrine disruptor validation study, testing CCI MCF-7 cell proliferation assays. KFDA's final report was submitted to ICCVAM in December 2010. The Korean Institute of Technology participated in a JaCVAM-led international validation study of the *in vitro* alkaline Comet assay test system. Data on four coded chemicals were submitted to JaCVAM in February 2011.

The director of KoCVAM is currently serving as a member of the NICEATM-ICCVAM endocrine disruptor study management team and as a consultant to the JaCVAM genotoxicity study validation management team. A Korean expert is a member of the NICEATM-ICCVAM scientific peer review study panel for *in vitro* endocrine disruptor tests. Another Korean expert has been nominated to serve on the ECVAM Science Advisory Committee peer review panel. The KFDA is also participating in a World Health Organization-led collaborative study of an *in vitro* method for specific toxicity of pertussis vaccine. Dr. Sohn reported on several pertinent research projects being pursued in Korea, including an ongoing exploratory study of the

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BrdU:LLNA-FC, a planned pre-validation study of that assay, and an ongoing exploratory study of the BCOP eye irritation assay.

In other activities, KoCVAM recently published a handbook on the validation of alternative methods in Korea. Dr. Sohn also described an international colloquium held in Seoul in August 2010, a second international symposium on global efforts for the validation of animal alternative tests held in Suwon in August 2010, a training workshop on alternative test methods also held in Suwon in August 2010, and a KoCVAM second Workshop titled *Understanding of International Validation Study* held in Suwon in May 2011.

She provided an overview of KoCVAM promotional activities, future activities planned by the group, including a third international symposium to be held at Hoseo University in July 2011 and a third training workshop on alternative test methods scheduled for November 2011.

### *Health Canada*

Michael Inskip, Environmental Health Science and Research Bureau, Health Canada, participated by telephone on behalf of Dr. David Blakey. Mr. Inskip provided an update on developments regarding Health Canada's role in ICATM since the 2010 SACATM meeting. He noted that in 2007, Canada had been invited to join ICATM. Although Canada has no validation center, it has contributed expertise to various stages of the validation process and test guideline development. The Environmental Health Science and Research Bureau, directed by Dr. Blakey, continues to coordinate Canada's input into ICATM. Within Health Canada, the Healthy Environments and Consumer Safety Branch, the Health Products and Food Branch, and the Pest Management Regulatory Agency are involved in the regulation of potentially harmful exposures.

Although there is no formal validation program in Canada, Health Canada contributes to pre-validation research, validation laboratory work, validation management committees and the peer review process. Health Canada contributes to method development, refinement, and validation through involvement with the ICCVAM Interagency Biologics Working Group. It also works with the Japanese Environmental Mutagen Society and is collaborating with ToxCast™ on mechanisms of action research. Health Canada is working to develop and evaluate alternative assays to the mouse safety test for residual pertussis toxin in vaccines and to develop alternative methods for testing of human biotherapeutics. Health Canada's Food Microbiological Safety group is collaborating with industry to evaluate and develop botulinum toxin detection assays in foods and biologics.

Mr. Inskip noted that in August 2011, Health Canada will host an ICATM meeting in Montreal, Quebec.

### *Japanese Center for the Validation of Alternative Methods*

Dr. Hajime Kojima provided an updated on recent JaCVAM activities. He briefly summarized the history of JaCVAM, which was organized in 2005.

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In February 2011, the Japanese Ministry of Health, Labour and Welfare was notified that data obtained for an alternative testing method approved by the JaCVAM Steering Committee could be used for the submission of quasi-drug applications, or for applications for ingredients for inclusion in the Standards for Cosmetics.

Dr. Kojima described a chart illustrating the regulatory acceptance system for new or revised test methods for quasi-drug and/or cosmetic products in Japan. He listed the test methods accepted by the JaCVAM regulatory acceptance board, which include the BCOP, ICE, LLNA:DA, LLNA:BrDU-ELISA, EPISKIN, *in vitro* skin corrosion testing, and *in vitro* cytotoxicity test methods. He listed test methods currently undergoing national or international peer review, e.g., the Bhas cell transformation assay and the LabCyte assay for skin irritation testing. Ongoing validation studies in which JaCVAM is participating along with several other international collaborators include the Human Cell Line Activation Test (h-CLAT), *in vivo/in vitro* Comet assay, STTA antagonist assay, and an assay for phototoxicity testing.

Dr. Kojima illustrated the history of JaCVAM's validation effort for the *in vivo* Comet assay, which has been in development since 2006. The next JaCVAM Validation Management Team meeting will take place in September 2011 in Kyoto. JaCVAM is also working on the STTA antagonist assay for endocrine disruptor screening, as well as a reactive oxygen species (ROS) assay for phototoxicity testing, which is working with the hypothesis that ROS may induce photochemical or toxic reactions. He described the high throughput ROS assay being developed, and the projected schedule for its validation study process, which should be completed by December 2011. In the assay's first validation study, three laboratories obtained identical results with 13 chemicals. This autumn, JaCVAM will start validation studies of *in vitro* immunotoxicity assays.

Dr. Kojima introduced two new projects to be undertaken in Japan—research and development of internationally leading hazard and test methods essential for Japan's new Chemical Management Policy, and the Agri-Health Translational Research Project, which explores agricultural solutions to medical problems. The first project will involve development of methods to obtain data on the possibility of the expression of toxicity on the basis of altered gene expression and development of cell assays to detect toxicities. The Agri-Health project will involve development of novel biomedical devices using animal-derived byproducts, leading to development of technologies for regenerative medicine and development of culture systems for alternative testing methods.

Dr. Niemi expressed SACATM's appreciation to Dr. Sohn and Dr. Kojima for traveling so far to attend the meeting, particularly to Dr. Kojima in light of the current difficulties in Japan related to the recent disasters.

### *European Centre for the Validation of Alternative Methods*

Dr. Sharon Munn, ECVAM *In Vitro* Methods Unit, participated by telephone and provided an update on recent ECVAM activities. ECVAM has recently completed validation studies for carcinogenicity, acute toxicity, and skin sensitization. The group currently has 10 validation studies in progress, involving 14 test methods, covering endpoints such as metabolism,

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endocrine disrupters, genotoxicity/carcinogenicity, eye irritation, ecotoxicity, and skin sensitization.

Dr. Munn described the ongoing ECVAM-led study on eye irritation, testing the EpiOcular™ eye irritation test and the SkinEthic™ human corneal epithelial model test. Thus far, 104 chemicals have been selected and are undergoing testing in three laboratories, with the testing phase scheduled for completion in July 2011, and peer review anticipated in March 2012.

Currently three ECVAM-led skin sensitization validation studies are in progress: the DPRA from Proctor & Gamble, the h-CLAT from Kao and Shiseido, and the MUSST from L'Oréal. The primary goal of the studies is to assess the reliability of the assays in terms of transferability and within/between laboratory reproducibility. The studies will also provide at least a preliminary view of the ability of the assays to discriminate between skin sensitizers and non-sensitizers.

In genotoxicity, Dr. Munn reported that the cosmetics industry group COLIPA, the European Cosmetics Association, is leading a validation study being supported by ECVAM to assess the EpiDerm™ test system. The objective of the study is to pre-validate the micronucleus test and the Comet assay in reconstructed human epidermis models. ECVAM is involved in the steering committee, is sponsoring one laboratory, and is providing statistical support.

ECVAM is also working with JaCVAM on a genotoxicity validation study designed to validate the *in vitro* comet assay. ECVAM is involved in the Validation Management Team for that study. Another ECVAM collaboration with JaCVAM is a carcinogenicity validation study, to validate the *in vitro* cell transformation assay in a BHAS42 cell line for the assessment of carcinogenic potential. ECVAM is part of the Validation Management Team for that study as well.

ECVAM is leading metabolism validation studies of two cytochrome P-450 induction-based metabolic-competent model systems, the cryoHepaRG® cell line and cryopreserved human hepatocytes. The goal of the studies is to assess transferability and reliability of the two model systems by challenging them with 12 coded chemicals, as well as to compare their abilities to detect cytochrome P-450 inducers and non-inducers.

For reproductive toxicity, ECVAM is leading a validation study of the MELN-ER TA assay. The study's objective is to assess the method in view of future incorporation into a testing strategy for detecting endocrine-active compounds.

Ecotoxicity studies include an ECVAM-led validation study of the Zebrafish embryo toxicity test and the *in vitro* trout S9 assay for fish bio-concentration testing.

Dr. Munn reported that ECVAM has had three test methods approved by OECD since October 2010 (1) the rLLNA for skin sensitization, (2) ICCVAM-ECVAM-JaCVAM harmonized LLNA performance standards, and (3) Test Guideline 439 issued on three *in vitro* skin irritation tests. Additionally, ECVAM has proposed test guidelines for two cell-based assays for eye irritation: the Fluorescein Leakage assay and the CM assay. ECVAM has also proposed an update to OECD TG 437 to allow the use of BCOP for the identification of UN GHS/EU CLP "non-irritants."

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During the 2010-2011 period, ECVAM made four full test submissions and nine test pre-submissions altogether. Also, four earlier test pre-submissions have been followed by an invitation to prepare for full test submission.

Dr. Munn said, with regard to the 2013 marketing ban deadline under the Cosmetics Directive, ECVAM, together with 39 stakeholder-nominated experts, produced a technical report summarizing the status and prospects of alternative methods for the endpoints of repeated-dose toxicity (including skin sensitization and carcinogenicity), toxicokinetics, and reproductive toxicity. The report was published in the *Archives of Toxicity* and on the European Commission website. It detailed the current status of test methods and outlined future prospects for alternatives.

ECVAM also recently participated in a post-validation workshop on the 3T3 NRU method for detecting phototoxicants, in conjunction with the European Federation of Pharmaceutical Industries and Associations. The workshop was convened in response to reports of a high rate of false positives with non-topical compounds.

There was a new directive on the protection of laboratory animals in the European Union (EU) last year, which enshrined the principle of the 3Rs in EU legislation. It also recognized ECVAM as an EU Reference Laboratory, and asked EU member states to nominate laboratories for validation studies. ECVAM will set up a European network based upon those nominations.

ECVAM has established an ECVAM Scientific Advisory Committee, which issued its first opinion in February 2011. The newly established ECVAM Stakeholder Forum held its first meeting in May 2011. May 2011 also saw the first meeting of the PARERE network, which has been set up to provide member state single points of contact for preliminary assessment of regulatory relevance. The network of suitable laboratories for validation will be set up this year. There is also a call out for experts to participate in an ECVAM Expert Pool, for experts to be invited for small advisory contracts.

Dr. Munn described a successful conference held by ECVAM in May 2011 in Varese, Italy, the Third International Conference on Alternatives for Developmental Neurotoxicity (DNT3).

## **XII. Closing Remarks and Adjournment**

Dr. Bucher expressed his appreciation to SACATM and the ICCVAM members for their participation in the meeting, and said he would benefit from their advice.

On behalf of NICEATM and the ICCVAM agencies, Dr. Stokes thanked the SACATM members for their comments, which he said are taken very seriously and will be enormously helpful as efforts move forward. He also thanked the NICEATM staff and ICCVAM representatives who had participated in the meeting.

Dr. Niemi again thanked departing SACATM members Drs. Meyer, Brown, Wnorowski, and Corcoran for their service. Dr. White stated the next meeting would be held September 5 – 6, 2012. Dr. Niemi then adjourned the meeting.