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Meeting of the Scientific Advisory Committee  
on Alternative Toxicological Methods  
June 25 – 26, 2009

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

Background materials and presentations for the SACATM meeting are available on the SACATM meeting web site (directly at <http://ntp.niehs.nih.gov/go/7441> or <http://ntp.niehs.nih.gov/> see “Advisory Board and Committees”).

3Rs	Replacement, reduction, refinement (causing less pain and distress) in the use of animals for toxicological testing
AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care
ATP	adenosine triphosphate
DACLAM	Diplomate, American College of Laboratory Animal Medicine
AMPC	antimicrobial cleaning product
ATSDR	Agency for Toxic Substances and Disease Registry
BrdU	bromodeoxyuridine
BCOP	Bovine Corneal Opacity and Permeability
BRD	background review document
CDER	Center for Drug Evaluation and Research
CM	Cytosensor Microphysiometer®
CPSC	Consumer Product Safety Commission
CRO	contract research organization
DA	Daicel Adenosine Triphosphate
DOD	Department of Defense
DOT	Department of Transportation
ECVAM	European Centre for the Validation of Alternative Methods
ELISA	enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
ESAC	ECVAM Science Advisory Committee
EU	European Union
FDA	Food and Drug Administration
FYP	NICEATM-ICCVAM Five-Year Plan
GHS	Globally Harmonized System
GPMT	guinea pig maximization test
h-CLAT	Human Cell Line Activation Test
HET-CAM	Hen’s Egg Test – Chorioallantoic Membrane
HSUS	Humane Society of the United States
IACUC	Institutional Animal Care and Use Committee
ICCR	International Cooperation on Cosmetics Regulations
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ICATM	International Cooperation on Alternative Test Methods
ICE	Isolated Chicken Eye

Minutes from the June 25 – 26, 2009 SACATM Meeting

IIVS	Institute for <i>In Vitro</i> Sciences
ILS	Integrated Laboratory Systems, Inc.
IRE	Isolated Rabbit Eye
JaCVAM	Japanese Center for the Validation of Alternative Methods
LLNA	Local Lymph Node Assay
LVET	low volume eye test
MOC	Memorandum of Cooperation
NCI	National Cancer Institute
NCTR	National Center for Toxicological Research
NICEATM	National Toxicology Program 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
OECD	Organisation for Economic Cooperation and Development
PETA	People for the Ethical Treatment of Animals
PI	principle investigator
REACH	Registration, Evaluation, Authorization, and Restriction of Chemical Substances
SACATM	Scientific Advisory Committee on Alternative Toxicological Methods
SI	stimulation index
SOT	Society of Toxicology
STTA	Stably Transfected Transcriptional Activation
TMER	test method evaluation report
TSCA	Toxic Substances Control Act
USDA	US Department of Agriculture
VAM	validation of alternative methods organization
WG	working group

## II. Attendance

SACATM met on June 25 – 26, 2009, at the Hilton Arlington, 950 North Stafford St., Arlington VA 22203. The following individuals attended the meeting:

### SACATM

James Freeman, PhD, ExxonMobil  
Biomedical Sciences, Inc., *Chair*  
Frank Barile, PhD, St. John's University  
Karen Brown, PhD, Pair O'Docs  
Enterprises  
Marilyn Brown, DVM, DACLAM, Charles  
River  
Grantley Charles, PhD, Allergan  
George Corcoran, PhD, ATS, Wayne  
State University

Helen Diggs, DVM, DACLAM, Oregon  
State University  
Marion Ehrich, PhD, VA-MD Regional  
College of Veterinary Medicine  
Donald A. Fox, PhD, University of  
Houston  
Steven R. Hansen, DVM, MS, MBA,  
DABT, ABVT, American Society for  
the Prevention of Cruelty to Animal  
Daniel Marsman, DVM, PhD, Procter &  
Gamble

Sharon A. Meyer, PhD, University of Louisiana at Monroe  
Gary Wnorowski, MBA, LAT,  
Eurofins/Product Safety Laboratories

**Liaison Representatives**

Joachim Kreysa, PhD, ECVAM  
Hajime Kojima, PhD, JaCVAM

**ICCVAM Primary Representatives**

George Cushmac, PhD, DOT  
Jodie Kulpa-Eddy, DVM, USDA,  
*ICCVAM Vice-Chair*  
Suzanne Fitzpatrick, PhD, DABT, FDA  
Pertti Hakkinen, PhD, National Library of Medicine  
Tina Levine, PhD, EPA  
Moiz Mumtaz, PhD, ATSDR  
Paul Nicolaysen, VMD, NIOSH  
RADM William Stokes, DVM, DACLAM,  
*NIEHS, NICEATM Director*  
Marilyn Wind, PhD, CPSC, *ICCVAM Chair*  
COL Peter Schultheiss, DVM, DACLAM  
DOD  
Margaret Snyder, PhD, NIH

**Other ICCVAM Representatives**

Paul Brown, PhD, FDA/Center for Drug Evaluation and Research  
Kristina Hatlelid, PhD, CPSC  
Raj Chhabra, PhD, DABT, NIEHS  
Vasant Malshet, FDA/Center for Devices and Radiological Health  
Richard McFarland, MD, PhD,  
FDA/Center for Biologics Evaluation and Research  
Jill Merrill, PhD, FDA

**Invited Speakers**

Gerald Gebhart, PhD, University of Pittsburgh  
A. Wallace Hayes, PhD, Harvard University  
Robert Kavlock, PhD, EPA

Michael Luster, PhD, West Virginia University

**NIEHS/NIH Staff**

Linda Birnbaum, PhD, DABT, ATS  
John Bucher, PhD, DABT  
Sally Fields  
Debbie McCarley  
Mary Wolfe, PhD  
Lori White, PhD, PMP (*Designated Federal Official*)

**Other Federal Staff**

Donnie Lowther, FDA  
Vasant Malshet, FDA  
Ying Huang, FDA  
Penelope Rice, FDA  
Martin Robl, PhD, FDA

**Image Associates Staff**

John Maruca

**ILS (NICEATM support contractors)**

David Allen, PhD  
Elizabeth Lipscomb  
Judy Strickland, PhD, DABT

**TeamPSA (station support contractor)**

Joseli Hagemann

**Public**

Rodger Curren, PhD, IIVS  
Julia Dady, Ecolab  
Kim Ehman, PhD, TRS  
Megha Even, TSG, Inc.  
Dmitry Gazarian, St. John's University  
Michael Jones, Strategic Diagnostics  
Sue Leary, Alternatives Research & Development Foundation  
Ann-Marie Matei, St. John's University  
Pat Rizzuto, BNA  
Martin Stephens, PhD, HSUS  
Sherry Ward, PhD, Biotred Solutions  
Neil Wilcox, PhD, FDA  
Kate Willett, PhD, PETA

### **III. Welcome and Introductions**

Dr. Freeman, SACATM chair, called the meeting to order at 8:30 A.M. Individuals in the room introduced themselves. Dr. Bucher, NTP Associate Director, welcomed the attendees and thanked them for participating. He looked forward to the reports from EPA and USDA and to discussions about ICCVAM's progress and new plans. He congratulated those involved in the Memorandum of Cooperation (MOC) for the International Cooperation on Alternative Test Methods (ICATM). He said it has been an exciting year and we may be seeing into the future of toxicology. The "pieces are now in place," catalyzed by the National Research Council (NRC) Report Toxicity Testing in the 21<sup>st</sup> Century to transform hazard assessment and risk assessment. Dr. Jodie Kulpa-Eddy, ICCVAM vice-chair, thanked the members of SACATM on behalf of the 15 member agencies of ICCVAM and looked forward to receiving advice from SACATM. Dr. White, Designated Federal Official, read the conflict of interest statement for SACATM.

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

#### **A. Presentation**

Dr. Stokes, NICEATM Director and ICCVAM Executive Director, welcomed everyone on behalf of ICCVAM and NICEATM and thanked SACATM. He said it has been a very busy year and acknowledged the hard work of ICCVAM, the ICCVAM working groups (WGs), and NICEATM. He pointed out the translational role of ICCVAM in moving new science and technology from the bench to tests that can be used for regulatory decision-making. He reminded SACATM of ICCVAM's mission to promote and facilitate the validation and regulatory acceptance of new, revised, and alternative methods that will ensure the continued protection of human health, animal health, and the environment while reducing, refining, and replacing animal use where scientifically feasible. Dr. Stokes reviewed the areas of emphasis of the NICEATM-ICCVAM Five-Year Plan (FYP): priority test method activities, application of new science and technology, partnerships, and international cooperation and harmonization. A new website page highlights milestones and tracks progress and activities. The ICATM MOC was signed at NIH on April 27, 2009, by Dr. Linda Birnbaum, Director, NIEHS and NTP; Dr. Nishijima, Director, National Institute of Health Sciences, Japan; Dr. Elke Anklam, Director, Institute of Consumer Protection and Health, Joint Research Center, European Commission; and Dr. David Blakey, Director, Health and Safety Bureau, Health Canada. ICATM provides a framework for enhanced international cooperation, collaboration, and communication with the goal to accelerate international adoption of scientifically valid alternative test methods.

Dr. Stokes reviewed the status of NICEATM and ICCVAM activities and progress with regard to alternative test methods for allergic contact dermatitis, ocular safety testing, acute toxicity, *in vitro* endocrine disruptor assays, genetic toxicity, dermal safety assessment, and biologics. He noted that more detailed presentations would be provided in separate agenda items summarizing recent regulatory acceptance of

alternative methods, two peer review meetings, and the implementation plan for the NICEATM-ICCVAM Five-year Plan. He then discussed NICEATM-ICCVAM's participation in the Society of Toxicology (SOT) Annual Meeting and the upcoming 7<sup>th</sup> World Congress on Alternatives and Animal Use in the Life Sciences.

## **B. Discussion**

Dr. Marilyn Brown asked if ICATM would focus on refinement. Dr. Stokes said all 3Rs (reduce, refine, and replace) would be addressed in the ICATM activities, adding that where animals still must be used, their use should be as humane as possible, which includes alleviating or reducing pain and distress to the extent possible. Dr. Charles asked about WGs. Dr. Stokes said NICEATM-ICCVAM has always used WGs, and the ICATM participating organizations will continue to be invited to identify liaison members to serve on each WG. Agency scientists with expertise in specific areas help to shepherd the test methods through the ICCVAM test method evaluation process. They review the background review documents (BRDs) to make sure they are sufficiently complete before becoming public and formulate the questions for peer review panels, and the initial draft ICCVAM recommendations. Dr. Barile asked about communication in the WGs. Dr. Stokes answered that holding teleconferences can be challenging due to the international participation, but they have been very effective. The co-chairs of the WGs are typically designated to serve on relevant validation management groups for other validation centers, so if the validation study is something the WG is addressing, the members typically would be invited to participate in face-to-face meetings of the validation management group.

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

### **A. Presentation**

Dr. Stokes discussed three different types of alternative test methods that have been accepted during the past year. All US agencies accepted the ICCVAM recommendations for four *In Vitro* Ocular Toxicity Test Methods for Identifying Severe Irritants and Corrosives in 2008. The four assays are: Bovine Corneal Opacity and Permeability (BCOP), Isolated Chicken Eye (ICE), Isolated Rabbit Eye (IRE), and Hen's Egg Test - Chorioallantoic Membrane (HET-CAM). The BCOP and ICE were recommended and accepted for regulatory safety testing, and were adopted internationally by the OECD the prior week. They are the first validated *in vitro* alternative test methods adopted for worldwide regulatory use and will likely reduce animal use for eye safety testing by 10 percent or more. He said the Organisation for Economic Cooperation and Development (OECD) adopted these methods quickly due to the comprehensive analyses provided by the ICCVAM Test Method Evaluation Report (TMER), BRDs, and peer review panel reports. The BCOP and ICE test methods should be used in a tiered-testing strategy, and always considered before using rabbits for ocular safety testing. Positive substances can be classified as ocular corrosives or severe irritants without the need for animal testing. This provides for animal reduction and refinement. ICCVAM recommended that users should submit data to NICEATM to support expansion of the validation database and generation of a histopathology database. This information would be used to better characterize the

usefulness and limitations of these methods, and determine whether histology could improve the accuracy of the test methods.

All US agencies endorsed the ICCVAM recommendations for *In Vitro* Cytotoxicity Test Methods for Estimating Starting Doses for Acute Oral Systemic Toxicity Tests. Data from the tests will not be used for regulatory decisions, but can be used to estimate starting doses for required acute oral toxicity studies. The methods will contribute to animal reduction and refinement; however, they are currently not sufficiently accurate to predict acute oral toxicity for the purpose of regulatory hazard classification. ICCVAM recommended that users submit data to NICEATM to expand the *in vitro* database so it can be further evaluated for usefulness and limitations. A proposed guidance document will be forwarded to OECD for consideration.

US agencies endorsed the ICCVAM recommendations for five *In Vitro* Pyrogen Test Methods Proposed for Assessing Potential Pyrogenicity of Pharmaceuticals and Other Products. ICCVAM recommended that these methods always be considered for use to detect Gram-negative endotoxin in human parenteral drugs before using animals for pyrogen testing and should be used where determined appropriate. Recommendations were provided for further research, development, and validation activities to improve their usefulness and broaden their scope of use and to confirm the extent that these methods can identify other pyrogenic substances in addition to Gram-negative endotoxin. There was international acceptance of the methods by the European Pharmacopoeia in March 2009.

Dr. Meyer asked if the regulatory agencies had enough experience with alternatives that they feel they could meet their mandated responsibilities to use the alternatives as stand-alone tests. Dr. Stokes said FDA has already accepted an *in vitro* pyrogen test for use with pharmaceuticals. Some agencies do not require submission of data, so they lack the experience of industry. The agencies have more experience with *in vivo* alternatives like the Up-and-Down Procedure (UDP) and the Local Lymph Node Assay (LLNA). Dr. Levine said EPA has been accepting UDP or limit tests for acute toxicity. She stated that more LLNAs have been submitted to the EPA, but there are questions about its use for pesticide formulations, which are mixtures. She added that EPA does not require companies to submit data if they judge a chemical to be category 1 for eye or skin irritation; EPA accepts a self-certification for category 1 substances. She was unsure about the use of BCOP. Test results do not provide the full labeling information, so the tests haven't been used as much. She said in the current regulatory scheme animal testing is necessary, so the alternatives are just additional tests. Mr. Wnorowski asked if EPA were accepting LLNA data for pesticide mixtures and Dr. Levine said yes.

Dr. Karen Brown asked who submits data to NICEATM. Dr. Stokes said companies could submit data, which are often generated by contract research organizations (CROs). However, NICEATM and ICCVAM do not accept confidential business information because of the handling requirements to assure confidentiality, and the limited usefulness of data that cannot be provided to the public and peer review panels for evaluation. Rather, NICEATM ask companies to code the data so they can be put in the public domain. Dr. Barile asked about contradictions in the endorsement of

pyrogenicity testing between ICCVAM and the ECVAM Science Advisory Committee (ESAC). Dr. Stokes said the ESAC's recommendations also include caveats that the test must be validated on a product-specific basis, consistent with the ICCVAM recommendations and FDA requirements. When used for pharmaceutical testing, companies must demonstrate that pyrogen contaminants can be detected in products without the product itself interfering with the assay. Dr. Barile said ICCVAM has been more conservative in recommending the LLNA and ocular tests than ECVAM and JaCVAM. Dr. Stokes said in the future there should not be inconsistencies because the groups are working together earlier in the process, during validation, peer review, and development of recommendations. He recognized that there may be differences, but ICCVAM, JaCVAM, and ECVAM are committed to working out the differences. If differences cannot be resolved, then all parties will be provided the basis for the differences. It is important for regulators to understand that the validation organizations do not make regulatory decisions; they make recommendations based on the science. Dr. Kreysa added that the validation organizations have a shared interest to provide scientific justification for positions stated in the validation studies; however, the countries' regulators work in somewhat different contexts. Dr. Stokes said under ICATM, the BRDs and independent peer reports used by any of the validation organizations would become publicly available, which has not been the case in Europe previously.

## **B. SACATM Discussion**

Dr. Barile, a lead discussant, commented that the work with international partners is coming to fruition and agreed with using a careful approach. ICCVAM has come a long way in making the tests known to various agencies. He said some of the tests are still in their infancy, but the process should be promoted and encouraged.

Dr. Corcoran, a lead discussant, said it was an honor to be appointed to SACATM. He suggested using a bottom-up approach with focus groups to engage Institutional Animal Care and Use Committee (IACUC) chairs and study directors to gain greater acceptance of alternative methods. It is important to know where IACUCs stand and to get feedback from them. Obtaining data from industry is difficult, but very important. He agreed with Dr. DeGeorge's comments from the last meeting regarding data mining, retrospective analyses, use of coded samples, and non-disclosure agreements. He asked what role ICCVAM could play in the reauthorization of the Toxic Substances Control Act (TSCA) and revision of the *NIH Guide for the Care and Use of Laboratory Animals* ("Guide").

Dr. Hansen, a lead discussant, asked if agencies offer incentives or expedited reviews to encourage use of alternative methods. Dr. Levine said the tests must give the agencies what they need for regulation. EPA has a pilot program with the antimicrobial cleaning products to get additional data for potential use in making labeling decisions. Mr. Wnorowski, a lead discussant, said regulatory acceptance is the real issue. Contract laboratories are aware of opportunities to use alternative methods. His company had difficulty obtaining confirmation that EPA is accepting LLNAs. He mentioned ICCVAM-recommended oral toxicity testing using the UDP, which agencies accept and which has been very successful. There has been some hesitancy for



acceptance of other alternative methods. He said if agencies do not accept methods, companies would be unwilling to implement them.

Dr. Charles said agencies have a great impact on getting new methods in use and promoting them. He thought most sponsors would agree to an *in vitro* pyrogenicity test in lieu of the rabbit pyrogenicity test if the agency reviewer would recommend it. Hands-on workshops at laboratory animal science meetings would increase awareness of alternative methods for study directors, veterinarians, and technicians. He said companies must see the merit of submitting data and would be most comfortable if coded data were submitted through a consortium of stakeholders for consideration by ICCVAM.

Dr. Corcoran asked about IACUCs requiring justification for research laboratories not using approved and validated alternative methods and whether there had been any progress since last year's meeting on this topic. Dr. Diggs said there is a requirement by IACUCs to minimize the number of animals used in research. If validated alternative methods would reduce, replace, or refine animal usage, the IACUC would require justification from the principal investigator for not using the alternatives. Dr. Brown added that agencies also need to require justification for not using alternatives. IACUCs, because of their other duties, cannot be expected to be knowledgeable about all areas of science, so the scientists must be aware of alternatives. She suggested greater outreach by electronic distribution of information regarding acceptance of alternative methods to IACUC chairs and scientists. CROs fear delays and wasted expense if alternatives are used and then not accepted by agencies. Dr. Freeman agreed that there could be a singular avenue for communication with IACUC chairs and scientists to both provide and collect information on compliance and utilization. Mr. Wnorowski said a practical point is whether the agencies will accept the studies. He said there is a clarification to the Animal Welfare Act stating that if a regulatory agency does not accept an alternative method, it does not have to be used. Dr. Marilyn Brown agreed that feedback from agencies is needed regarding acceptance of methods.

Dr. Kulpa-Eddy asked for specific venues, beyond the ICCVAM listserv and American Association for Laboratory Animal Science to use for disseminating that information. Some of the tests are very new, so examples of acceptances would encourage their use. Dr. Stokes said ICCVAM has been distributing more information to professional organizations. He suggested having IACUCs search the ICCVAM website for alternatives since the information on regulatory acceptance by each agency is included there. A follow-up with the agency might be required if any ambiguity were found in the agency's response. Dr. Kulpa-Eddy added that the USDA Policy 12 is being revised and ICCVAM would be added as an additional resource for alternatives. Dr. Karen Brown said some industries might not be aware of ICCVAM and the requests for data. She said archival data was present at USDA and at companies, but it is difficult to collect, organize, blind, and submit. Additional education of IACUCs about ICCVAM is critical. Dr. Barile said he would like to have ICCVAM encourage contract research organizations and other stakeholders to do more histopathology.

Dr. Marilyn Brown asked about the necessity for validation of alternative tests every time a study is done for a compound, which would increase animal usage. Dr. Stokes clarified that product-specific validation is only applicable to the *in vitro* pyrogen tests. Once a method is validated for a specific product, it does not need further validation. Dr. McFarland added that the standard practice for pyrogenicity testing in the pharmaceutical industry is similar in the United States and Europe. The FDA interacts with ICCVAM, sister regulatory agencies, and the pharmaceutical industry. Dr. Marsman encouraged ICCVAM to include in the databases information on the mitigation of pain and distress and the use of more humane endpoints. He considered that information underrepresented in terms of methodologies and approaches; the information would be an asset to both IACUCs and researchers. Dr. Meyer suggested ICCVAM's website be included on the list of guidance references that study directors must use. She said the culture must be changed from the bottom up and recommended incorporating information about alternatives in undergraduate and graduate training.

## **VI. National Research Council Report: *Recognition and Alleviation of Pain in Laboratory Animals***

### **A. Presentation**

Dr. Gerald Gebhart, chair of the NRC Committee, presented an overview of the findings and recommendations in the report: *Recognition and Alleviation of Pain in Laboratory Animals*. Its objective was to update a 1992 report and incorporate, where possible, evidence-based knowledge and advice. The NRC also published *Recognition and Alleviation of Distress in Laboratory Animals* in 2008. An appendix in the pain report describes different models of pain. The goal of the report was to inform IACUC members, investigators, animal care staff, and others regarding the basis of animal pain, the recognition/evaluation of pain, and the means for reducing and alleviating pain.

He explained that pain is an aversive state experienced by all mammals and probably all vertebrates. Pain should be limited to that which is unavoidable. It may be induced deliberately when pain is the subject of study, is inferred from behavior, and is a cascade of physiological, cognitive, immunological, and behavioral events. Pain is difficult to assess and depends on a structured clinical examination and knowledge of normal animal appearance and behavior. He said reliable, broadly applicable pain assessment tools are lacking as well as the funding to develop those tools. Observing animals' response to analgesics can refine clinical assessment. Anticipating post-procedural pain intensity is central to prevention, but effective pain management is limited by lack of knowledge of drug effects and doses. Pilot studies can be invaluable to determine earlier and more humane endpoints. He said there is little funding in the US to develop and validate strategies to refine procedures and reduce numbers of animals.

He hoped the report would be used to increase awareness about the absence of useful information and the need for funding of studies to gain knowledge and validate procedures and drug effects. The report will provide information about painful procedures and conditions, the ontogeny of pain (especially in the postnatal period), methods for assessing pain, strategies for managing pain, guidelines for establishing

humane endpoints, pain models, and a summary of relevant policies. He said there is no way to objectively assess pain or to define what severe pain is in animals. Reliability of clinical observations is essential. The report suggests pilot studies to lessen the negative impact of pain and identify objective endpoint criteria. Observation of behavior by experienced persons and animal weight loss are two ways to assess pain.

Dr. Marilyn Brown asked about preemptive pain control. Dr. Gebhart said there is no good current evidence to validate the use of a preemptive strategy when an appropriate post-surgical strategy for management of pain is used. Dr. Levine asked about the background of the committee and how fish and snakes perceive pain. Dr. Gebhart said some behaviorists were included on the committee. He said the evidence, using learned behavior, is not strong that fish can perceive pain. Dr. Gebhart described the expertise of the panel and said the evidence available on pain is very poor for animals other than rodents.

### **B. Public Comments**

Dr. Kate Willett, People for the Ethical Treatment of Animals (PETA), said PETA sent all IACUC chairs a packet with information about alternatives. PETA is interested in having training courses for IACUC chairs that would include information about ICCVAM-recommended methods. Dr. Alka Chandra, her colleague at PETA, recently wrote a paper that catalogues work by Dr. Flecknell and colleagues regarding the use of analgesics and anesthesia in rodents. For many procedures that are expected to be painful, analgesics and anesthesia are used less than 50% of the time. A concern among some researchers is that analgesics and anesthesia will interfere with experimental results; however, pain and stress also interfere with results. Some of the conclusions of the report could get researchers to start using analgesics and anesthesia for rodents, which are not covered under the Animal Welfare Act.

### **C. SACATM Discussion**

Dr. Corcoran, a lead discussant, said recognition and alleviation of pain were a very important topic. He thought the report's focus on birds, amphibians, and fish was overdue and worthwhile. The report points researchers in the right direction and may help renew future efforts to broaden the evidence base for these test animals. He asked about the current guidelines for pain relief. He said IACUC is the front line for alternative methods and for managing pain and distress. In his experience, the committees are highly variable in quality, may be biased, and are very dependent on the guidance from the veterinarians. He expressed concern about low adoption of alternatives and asked if there were advocates for alternatives on IACUCs. He proposed having regional ICCVAM experts on alternatives that could provide the latest information on the 3Rs. He mentioned the sometimes-adversarial relationship between the principal investigators and IACUCs and suggested an early intervention to change that dynamic and transform the culture.

Dr. Diggs, a lead discussant, said pain and distress have the potential to affect research outcomes and sometimes might have the opposite effect of causing additional animals to be used. It must be a priority to fund pilot studies and targeted pain research. More validated information is needed; anecdotal discussions and subjective observations are

not enough. Veterinarians who provide advice and information about pain and distress and who take action to minimize pain need additional training on recognition and alleviation of pain. She proposed a training program that includes multiple veterinary specialties such as laboratory animal medicine, anesthesiology, behavior, and pharmacology. The program should include graduate training and would enhance the ability to review safety testing methods. IACUCs must have the power and tools or the effort will not move forward. A bottom up approach is needed, with early career training in pain management. The desired outcome of the training program is the enhanced dissemination of knowledge. She asked if ICCVAM has been involved in the NRC update of the *Guide*. Dr. Stokes responded that ICCVAM submitted documents, such as workshop proceedings and relevant publications on humane endpoints and pain and distress, for consideration by the NRC committee.

Dr. Hansen, lead discussant, agreed with the comments about the importance of communicating with IACUCs and educating investigators. It is essential to further influence IACUCs and to identify endpoints relevant to the alleviation and mitigation of pain.

Dr. Marsman, a lead discussant, commended the authors and said the reports would set the framework for greater emphasis on addressing alleviating pain and distress in testing, research, and educational environments. The report had the correct focus and it should encourage ICCVAM, NICEATM, and the sponsoring agencies to advocate that pain and distress be addressed in all studies. Modeling and encouraging this behavior would help to establish it as normative and serve to fill in the gaps in the baseline database of studies conducted with appropriate analgesia and anxiolytics. The priorities of ICCVAM-NICEATM should include efforts to: identify funding for pain research, encourage research for all 3Rs, target the most painful and distressing procedures for replacement, target research into therapeutic and non-therapeutic approaches to minimize pain, provide a database of pain information for researchers, and advance refinement with the exploitation of humane endpoints (he alluded to Chapter 5 of the report). He highlighted some items in the report including: animal models with unforeseen complications that lead to pain and distress, the continuum between pain and distress, conditions in the necropsy suites for both animals and animal handlers, the distinction between nociception pain, animal models that eliminate pain, the use of tranquilizers, therapeutic regimens that may mask good pain and lead to greater destruction, and the potential for nonsteroidal drugs to undermine the interpretation of studies.

Dr. Marilyn Brown, a lead discussant, said IACUCs are supportive of pilot studies, but investigators often cannot budget for them. Industry is more likely to do pilot studies than academia. She said it was important to publish results when anesthetics, analgesics, and non-therapeutics are used that do not affect the outcome of the study. Any outreach communications should highlight information on the use of anesthetics, analgesics, and non-therapeutics. It is a regulatory requirement for a researcher to consult a veterinarian in the development of a study if there is the potential for pain and distress. There are receptive audiences at meetings for this information. Agencies should ask questions about pain management in the data submitted to them.

Dr. Ehrich asked about data on cats, dogs, and horses in veterinary teaching hospitals, which have protocols for managing pain. Dr. Gebhart responded that data on rodents are typically published in peer-reviewed literature, whereas there are few management strategies used in hospitals that are published. He agreed with SACATM's comments, said resources must be made available for controlled studies, and encouraged publication of data. Dr. Bucher said pain research is an area that falls through the cracks. There is a role for the toxicology portion of NIH to put forward funding mechanisms to allow pain research to be addressed in the academic community. He agreed to discuss the issue with Dr. Birnbaum. Dr. Snyder encouraged partnerships with the Dental Institute, where pain research had been occurring for years. She said the first step in protocol review is a discussion with and approval by the laboratory animal veterinarian, who offer suggestions on limiting pain and distress. She recommended further research on pheromones and stress responses. Dr. Snyder expressed optimism that information from both the pain and distress reports would be included in the updated *Guide*, which will be web-based and searchable.

Dr. Schultheiss said the institutional officials must be very involved in the animal care and use programs, be knowledgeable about policies, and hold IACUCs, veterinarians, and investigators accountable. ICCVAM should reach out to institutional officials because they have the ability to commit resources and leverage scientists' careers. Dr. Meyer expressed concern that dose responses could be shifted depending on the pain management strategy. She suggested that if pain were part of a response to the test article, and pain and distress were alleviated, then it might eliminate the toxicity of the chemical and shift the dose response to the right.

## **VII. Draft Implementation Plan (the Plan) for the 2008-2012 NICEATM-ICCVAM Five-Year Plan**

### **A. Presentation**

Dr. Fitzpatrick, FDA, explained that, in response to a directive from US House and Senate Appropriations Committees, NICEATM-ICCVAM, in partnership with relevant Federal agency program offices, created a FYP that built on the NTP Roadmap. The goal of the FYP was to advance alternative test methods of high scientific quality to protect and advance the health of people, animals, and the environment. The NTP Roadmap and the FYP are consistent with the recent National Academy of Sciences Report *Toxicity Testing in the 21st Century: a Vision and Strategy* and also build on current US laws, policies and regulations. She explained that cooperation is essential among ICCVAM agencies and its many stakeholders for implementation of the FYP.

Implementation activities address the four key challenges of the FYP: (1) identify priorities and conduct and facilitate activities in these areas, (2) identify and promote new science and technology, (3) foster regulatory acceptance and use of alternative test methods, and (4) develop partnerships. The four highest priority areas are biologics/vaccines, ocular toxicity, acute toxicity, and dermal toxicity. Dr. Fitzpatrick explained the basis for the high priority in each of the areas and then described planned activities and current progress. She also reviewed the progress made in other priority

areas including immunotoxicity, endocrine disruption, pyrogen testing, reproductive/developmental toxicity, and chronic toxicity/carcinogenicity testing.

She described some of the new science and technology in areas including high throughput screening, other animal species, computational approaches, biomarkers of toxicity, toxicology databases, and nanomaterials. ICCVAM is fostering acceptance and use of alternatives using an updated website that includes comprehensive information on its activities. Every ICCVAM agency is also encouraged to create a webpage to provide information about alternative methods accepted by that agency. ICCVAM is developing partnerships and strengthening interactions with its stakeholders by working with other national and international validation organizations (e.g., ECVAM and JaCVAM). She highlighted the MOC signed for ICATM in April 2009. She mentioned that a list of research projects in various agencies is included in the Plan and responded to some SACATM questions discussed earlier. She explained that pharmaceutical companies meet with FDA before they develop their preclinical screening so they can get information about what alternatives are accepted. FDA also reaches out to trade groups to spread information. At FDA, expedited reviews are used for life-threatening issues and incentives are offered for orphan drugs. She suggested an on-line survey through SOT to advance *in vitro* methods and mentioned that Johns Hopkins offers a certificate and provides on-line training in alternatives.

Dr. Marilyn Brown asked about the timeline for putting information regarding alternatives on agencies' websites and the possibility for ICCVAM to provide a format and infrastructure. Dr. Fitzpatrick said ICCVAM has provided an infrastructure. The Plan provides milestones and deadlines and a potential scorecard. Dr. Karen Brown asked about including in the upcoming workshop on alternatives for biologics testing a discussion about reducing pain in animal disease models and potential early endpoints. Dr. Barile asked if agencies could encourage companies to use non-animal alternatives to enhance the companies' image. Dr. Fitzpatrick responded that agencies could not do it individually, but nonfederal government organizations might. FDA's goal is to ensure that preclinical safety testing supports clinical trials.

Dr. Marilyn Brown discouraged use of the term, "not tested in animals," because it is misleading and asked about FDA publicizing its research on the website. Dr. Fitzpatrick said research would be promoted on FDA's website. Dr. Stokes said relevant Federal research is listed on ICCVAM's website and it will become better categorized. Dr. Fitzpatrick mentioned some new efforts at FDA such as the critical path program looking at biomarkers and surrogate endpoints that could be applied to animal testing. Dr. Karen Brown suggested better accessibility to information on agencies' websites such as an obvious link on the home page. She suggested extending the life of master references so host animal testing does not have to be repeated every five years and companies do not have to devote entire research groups to re-qualifying references. Some companies are returning to animal testing because it is less expensive and time-consuming than re-qualifying their master references every five years. Dr. Hansen asked how frequently pre-study meetings occur and if there were roadblocks or possible incentives. Dr. Fitzpatrick said meetings are not mandatory and companies are penalized only when they do something the agency does not agree with.

## **B. Public Comments**

Dr. Kate Willett, PETA, asked about a formal comment period for the Plan. Dr. Stokes responded that this meeting is the opportunity to provide public comment, but ICCVAM would accept comments any time. The Plan is a living/changing document that will be updated as priorities shift. Details of projects can be impacted by suggestions from stakeholders. Dr. Willett said many items in the Plan are encouraging. PETA would like to see less generic and more specific descriptions, especially of the priority areas. ICCVAM should articulate the state of the art for each area and how planned activities will build on it. ICCVAM should provide a description of the intended outcomes that tracks progress. She provided some suggestions for future workshops: (1) present the state of the art at the beginning of the workshop, (2) build on previous workshops, and (3) provide summaries and recommendations. Regarding peer reviews, she mentioned some procedural difficulties: (1) the panel does not have a comprehensive view of the subject being discussed, (2) the peer review panel misunderstands its charge, (3) panel members are unaware of the validation and acceptance procedures, the panel's role, and the ICCVAM process, (4) panel members are not provided background information on the current procedures and methods, (5) panel members have unreasonable expectations regarding the alternative methods, (6) experts and stakeholders present in the audience are not allowed to interact with the panel, and (7) panel members are not aware that they can ask questions. She suggested an orientation workshop for panel members to prepare them and a simple set of focused questions for review. She said the ocular review was supposed to be a quick review of a process for antimicrobials, but turned into something much larger and got bogged down. The EPA has initiated an independent pilot program to allow submission of data from non-animal studies. She said none of the methods in EPA's endocrine disruptor testing program have been validated by ICCVAM in ten years. ICCVAM has a limited number of resources and must rely on others for research. ICCVAM must be more efficient and work on smaller issues or the committee will become increasingly irrelevant because agencies will do their own validation work.

Dr. Stokes responded to Dr. Willett's comments on the peer review process. He said peer review panels undergo orientation by NICEATM-ICCVAM staff by teleconference before they begin review of the materials. The panel is provided documents that describe the process in great detail and attend an orientation session on the afternoon before the meeting to explain the procedures to be followed. Public comments are held after the panel deliberation, but before the panel votes on any of the deliberations, in order to allow the public to be aware of what the panel is thinking and to have an opportunity to comment on the deliberations before the panel reaches its final position. All panel members have the opportunity to query public commenters and generous time is allotted for public comment. During the ocular panel there were 10 public comment sessions. Test method developers were invited to be present and describe how to conduct and interpret the test methods being considered; one developer had three presentations. The panel chair instructed the panel that questions could be asked of the developers. ICCVAM has worked to refine the peer review processes over the past 10 years and strived to make sure that public comments are considered before the panel's final deliberations. ICCVAM is always open to suggestions to improve the

process. Dr. Stokes thanked Dr. Willet for her comments and hoped his remarks corrected some misconceptions about the process.

### **C. SACATM Discussion**

Dr. Meyer, a lead discussant, said ICCVAM did a very good job addressing the issues. She reiterated Dr. McClellans's comment that the primary goal is the protection of the health of humans, animals, and the environment. A data gap in the Plan is the need for mechanisms to ascertain concentrations; dose response information is needed. Comparisons between *in vitro* concentrations and blood concentrations are needed to allow biological modeling. Discussions on limitations of methods should include whether there is a lack of human data. Regarding the emphasis on partnering with other agencies, she cautioned that other agencies may have other priorities. Regarding new methods, when the UDP was developed, its reduced precision for LD<sub>50</sub> was accepted because EPA used LD<sub>50</sub> values largely for hazard classification categories. Earlier mechanistic endpoints are often continuous data, which can provide more robust statistics. She said a bottom up approach is essential.

Dr. Marsman, a lead discussant, agreed with Dr. Meyer. He suggested moving beyond acute endpoints and shifting to other endpoints that are more difficult to address such as repeat dose endpoints. Success criteria need to be clearly defined at the outset. Identification of false positives and negatives is difficult with alternatives when being compared to animal studies that are still treated as the gold standard. He supported nanotechnology, high throughput screening, computational approaches, databases, and quantitative structure-activity models to make better inferences from existing data and decrease animal usage. He suggested broader use of workshops earlier in the process to allow a more open dialogue. Partnerships with organizations like ECVAM are valuable going into phase II of the seventh amendment of the Cosmetics Directive.

Dr. Diggs, a lead discussant, said the Plan was a tremendous effort and she was impressed with the number of projects in the past year. She encouraged a focus on priorities and not doing too much, too fast.

Dr. Corcoran, a lead discussant, concurred with Dr. Diggs. He said the plan was an amazing effort and an overwhelming body of work. He saw a consistent effort to prioritize. He asked if a more hierarchical structure could develop as the Plan evolves, which would allow looking back at action items with specific milestones. He expressed confusion about acceptance being targeted for end users or agencies; if both, there should be two strategies. He said the plan read more like a progress report than a future implementation plan, with the focus on years one and two and little on years four and five. He said partnerships are "the lifeblood of ICCVAM" and he was completely impressed with the interagency cooperation, but felt the goals should be more concrete. He thought it important to harmonize with the NRC report *Toxicity Testing in the 21<sup>st</sup> Century* in the effort to move toward the use of more human tissues and cells. He suggested creating themes, such as human models, that would cut across the four challenges of the plan.



Dr. Marilyn Brown, a lead discussant, fully concurred with previous comments. She said refinement must be kept a priority and there are opportunities to make real improvements. She suggested using information technology as a crosscutting theme to provide more outreach to stakeholders and allow greater interactive participation in workshops. She suggested additional work utilizing biomarkers to identify permanent perturbations and develop humane endpoints. Regarding fostering acceptance, there must be acceptance at the agency level before there is acceptance at the user level. She urged dissemination of information about alternative use by agencies and was pleased by the incredible advances in partnerships.

Dr. Charles complimented ICCVAM on the plan, especially the WGs. He urged use of webinars to spread information about alternatives. Dr. Marilyn Brown added that the webinars could also be archived so that they could be viewed at a later date. Dr. Freeman said the topics being discussed at the meeting all related to the four areas in the FYP. Dr. Stokes thanked SACATM for its constructive comments. ICCVAM is very interested in computational toxicology and participates in an interagency computational toxicology colloquium that meets every six months. Regarding fostering acceptance, ICCVAM is aiming for acceptance at both agency and user level and this would be clarified in the Plan.

Dr. Mumtaz, ATSDR, said he appreciated comments by SACATM and expressed optimism for the future of the plan. He said methods development and regulatory acceptance are real challenges. He considered it important to continue the dialogue at public meetings as the plan is further developed, and he urged private industry to provide data.

Dr. Levine said most of ICCVAM's decisions have been qualified, i.e., the alternatives do not fully replace current tests, but can be used as substitutes or hazard category decisions in a tiered testing strategy. The current system of validating alternatives against standards like the Draize test makes it very difficult to create full replacements. She questioned what should be done from a regulatory perspective, because if tests are not full replacements, her opinion is that they will not be used. Dr. Marilyn Brown asked about strategies companies should use in dealing with regulatory agencies. Dr. Levine said agencies other than the EPA might have more flexibility in using ICCVAM-recommended methods. Dr. Birnbaum asked if the requirements for registration and labeling of pesticides could be changed to allow acceptance of tests that might not be total replacements. Dr. Levin said the EPA is reviewing its labeling policies, but ICCVAM reviews tests are based on the current labeling schemes. Dr. Stokes clarified that ICCVAM does not work on just replacements, but rather on tests that can be used as a substitute for some applicability domains, and on tests where positive results can be used to identify certain hazards for classification purposes. Dr. Freeman added that the ICCVAM-recommended tests should not be considered as one-for-one replacements, but rather a way of expanding the toolbox and in line with recommendations in *Toxicity Testing in the 21<sup>st</sup> Century*.

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

## **A. Presentations**

### *US Environmental Protection Agency*

Dr. Kavlock, EPA, said the EPA's work is addressing a number of challenges in the FYP. The Office of Research and Development's mission is to lead the translation of scientific advances to address problems of national and international importance relative to protecting human health and the environment. Current methods have been insufficient to assess new chemical hazards, so five years ago the EPA launched the National Center for Computational Toxicology (NCCT), with the goal to, "integrate modern computing and information technology with molecular biology to improve Agency prioritization of data requirements and risk assessment of chemicals." This work is a priority for the new administration. The EPA got input from the National Academies reports and workshops to create a strategic plan.

Dr. Kavlock described five projects the NCCT has underway to apply computational toxicology along the source to outcome continuum. ToxCast™ was launched in 2007 and has run over 500 assays on 320 chemicals for which there are developmental toxicity studies, multigenerational studies, and chronic bioassays. ToxCast™ will eventually assay ~10,000 chemicals, many of which currently have very little toxicity data. He provided an example of a traditional developmental toxicity study's results by presenting a heat map in the ToxRefDB of 283 chemicals for 19 target systems in rat and rabbit. Such endpoints are being used to anchor existing testing results to the results from the new technologies. The ToxCast™ assays cover 467 endpoints and include both traditional biochemical assays and cellular assays, many of which use human-derived cells or proteins. He explained that there are some important redundancies in the assays and showed some data and preliminary prioritization rankings for endocrine disrupting chemicals. He provided some pathway information for chlorpyrifos and organophosphates and shared some of the lessons learned in a recent NCCT data analysis summit for the Phase I ToxCast™ data. Phase II will expand to include human toxicants using chemicals supplied by pharmaceutical companies. The NCCT also has two projects developing virtual models of the liver and the embryo. These systems biology approaches were initiated in order to bring the high throughput testing results into better context for risk assessment. He described the development of the Tox21 community (a collaboration of the EPA, NIEHS and the National Humane Genome Research Institute) that is currently assembling a library of 10,000 chemicals that will undergo high throughput testing at the NIH Chemical Genomics Center. Tox21 has four WGs, pathways/assays, compounds, bioinformatics, and targeted testing. Tox21 has two virtual tissue projects, liver and embryo. He said the future state is to use high throughput technologies for exposure and hazard assessment to assist in design and prioritization of testing and monitoring.

### *US Department of Agriculture*

Dr. Kulpa-Eddy, USDA, said the Center for Veterinary Biologics (CVB), a small component of the USDA, is responsible for regulating veterinary biologics such as vaccines, bacterins, antisera, and other products of biological origin to ensure they are pure, safe, potent, and effective. The potency testing done by CVB is relevant to the FYP. In the 1960-70s all vaccines required vaccination and challenge of target species,

or surrogate laboratory animals, for serial release. Potency testing of modified live virus vaccines was replaced by quantification of the live organisms, which was a major step toward reducing animal use by about half. Since the 1980s, CVB has expanded testing to include both live viral and live bacterial vaccines. In 1997 the regulation was revised to include potency testing by relative antigen content, which could theoretically be used for many products with some caveats. Potential drawbacks to these *in vitro* tests, however, include measurement of limited numbers of antigens, inability to determine levels of active agent, interference by adjuvants, and inability to differentiate active from denatured antigen.

The USDA provided information on three potency test research projects to ICCVAM in conjunction with the development of the FYP, and provided updates on these:

- 1) *Clostridium haemolyticum* – a bacterial toxin that affects cattle and sheep and causes hemoglobinuria, jaundice, and death. The current testing uses a challenge vaccinate and up to 15 guinea pigs. A CVB Science Fellow, working from 2003-2007, developed monoclonal antibodies, but no further funding is currently available to continue this research.
- 2) *Leptospira serovars* – affects dogs and livestock and causes jaundice, fever, and kidney failure. The current testing is a challenge vaccinate test using 20 hamsters. The USDA has established a Standard Reference Bacterin that may be used for an *in vitro* potency assay.
- 3) *Rabies* – a virus that affects the central nervous system of all warm-blooded species and is almost always fatal. The current test is a challenge test with some earlier humane endpoints. Testing has been further refined using a scoring system. CVB submitted a proposal to develop an *in vitro* assay, which was found to have merit, but not funded. CVB is working on further refinements to the *in vivo* assay, participating in a collaborative study with European laboratories.

CVB has an on-going goal to reduce animals used for mandatory nine Code of Federal Regulations testing. They encourage manufacturers to submit alternative methods for animal potency assays.

## **B. SACATM Discussion**

Dr. Karen Brown, a lead discussant, was impressed by the data collected and mined by EPA. She asked how the testing would be applied in the real world. Dr. Kavlock said right now it is a research program and implementation will depend on the Congress providing funding for screening. He said all the testing EPA uses can be done by contract organizations. Dr. Karen Brown mentioned her early career experience with *Leptospira* testing and compared EPA and USDA's databases. Much of the historical data from companies making *Leptospira* vaccines were lost, so USDA must repeat all the host animal immunogenicity studies. She urged that all data be saved and mined, and that historical data be sought before a project is started. She was very supportive of USDA supplying master references and urged government and industry to work together to reduce the amount of interim stability testing of the master references. If a company has proven through repeat immunogenicity studies that a reference is stable for 15 – 20 years, the next reference they make should have a stability of 20 years.

NICEATM-ICCVAM should provide as much support as possible for development of *in vitro* assays for use as total replacements for vaccine testing.

Dr. Charles, a lead discussant, said the agency presentations were tremendously helpful and provided a good perspective of the overall global nature of the portfolio. He suggested increasing use of humane endpoints, mining data from current activities, and using WGs to feed back knowledge about alternative methods in an iterative process.

Dr. Ehrich, a lead discussant, said much activity is occurring and many agencies support high throughput screening developments. ToxCast™ is one way agencies can work together. The USDA and the FDA can work together on vaccines, but she was not clear about some of the other working relationships among agencies. She saw a lack of information on some of the newer products such as botanicals, probiotics, and herbals. She also asked about the use of remote telemetry and non-invasive imaging for more humane testing of vaccines. She concurred with the priority given to biologics.

Dr. Fox, a lead discussant, said both dermal and ocular testing need to move the focus away acute corrosives and overt injury to more to more long-term adverse effects, subacute effects, lower doses, and recovery. He was impressed by ToxCast™, but questioned how decisions were made regarding what is tested and the cell lines used for testing. He suggested ICCVAM-NICEATM could provide good input. He said toxicity is mediated at different levels (mRNA, protein, post-translationally), and metabolism must be considered. *In vitro* systems cannot test for maternal/fetal relationships, sensory-motor changes, cognitive development, and age-specific changes. It was essential to differentiate between adaptive and toxic responses. He suggested ways to increase the sensitivity of assays used in biologics testing. He supported the work in bioinformatics, *in silico* methods, and public data sharing. NICEATM should reach beyond the 15 Federal agencies and work for harmonization more globally. He said it is important to understand how concentrations in *in vitro* systems relate to biological doses. He expressed optimism for the future of the 3Rs. Dr. Kavlock noted that the NCCT is addressing the issues of cell types and metabolism in their assays. Biology is complicated, but much of toxicity is driven by a limited number of key events. There may be computational way to solve some problems. The EPA is committed to data release and makes databases available on its website. He mentioned an OECD WG on molecular screening approaches and invited interested parties to join. In the European Union (EU), a number of projects are being funded through the Seventh Framework to support computational research and enhance international coordination. Dr. Fox said the EPA should interact with ICCVAM-NICEATM to do studies to test cell systems with a more limited number of reference chemicals and then apply algorithms and network analysis across systems to identify the most sensitive systems. Dr. Kavlock said an issue in using a small number of chemicals is sparseness; i.e., the chance of getting a chemical that is active in an assay is very low.

Dr. Meyer, a lead discussant, concurred with previous comments. She said the ToxCast™ approach resembles pharmaceutical development. Pharmacokinetics and bioavailability apply to animals' adverse response to chemicals. ICCVAM and

ToxCast™ have different, but overlapping, priorities. She could not see how a gene expression assay would give metrics useful for ICCVAM's current priorities. She asked about the missing data for the 10,000 chemicals. Dr. Kavlock said information might be available from 28-day studies, but not from 2-year chronic bioassays or multigenerational studies. Dr. Meyer said, with the heat maps, there is the potential for identification of biomarkers with wide applicability across species. She asked about the use of differentiated stem cells. Dr. Kavlock said ToxCast™ is partnering with a group using mouse embryonic stem cells. Dr. Birnbaum added that guidelines for NIH-funded human embryonic stem cells use would be released on July 7. Dr. Fox said it should be possible to develop *in silico* methods for testing botulinum toxin. Dr. Meyer asked about using stimulus money to complete studies on biologics. Dr. Birnbaum said some NTP-supported contracts have gotten stimulus money for the Tox21 effort.

#### **IX. Welcome and Remarks by Dr. Birnbaum**

Dr. Birnbaum, NIEHS and NTP Director, welcomed attendees on behalf of NIH, NIEHS, NTP, NICEATM, and ICCVAM. She thanked SACATM members for their service and acknowledged ICCVAM representatives, NICEATM staff, and the international liaisons. She reviewed the public health role of NICEATM and ICCVAM in protecting, promoting, and advancing the health and safety of people, animals, and the environment by translating research advances and new technologies into scientifically valid safety testing methods for regulatory use. ICCVAM also serves a vital role in assisting agencies in meeting the requirements necessary for new test methods to be adopted for regulatory decision-making, which include adequate validation and determining that use of the new test method will provide equivalent or better protection than the existing test method. She highlighted some examples of ICCVAM's accomplishments and impact which include: (1) the endorsement or adoption of over 27 alternative methods including nine new test methods adopted by Federal agencies in the last year; (2) Federal agencies' acceptance of the first two *in vitro* test methods that identify substances that can cause blindness or other severe eye damage without using animals; (3) the recommendation that anesthetics, analgesics, and humane endpoints should always be used for ocular safety tests involving animals; and (4) establishment of and participation in ICATM. She mentioned the current and future endeavors outlined in the Plan and the ICCVAM Research and Development WG. Dr. Birnbaum closed by congratulating ICCVAM and NICEATM on their many accomplishments and by presenting letters of appreciation and certificates to the five members of SACATM who are completing their terms, Drs. Fox, Barile, Charles, Marilyn Brown, and Marsman.

#### **X. Report on the Independent Scientific Peer Review Panel on Alternative Ocular Safety Testing Methods**

##### **A. Presentations**

Dr. Merrill, FDA, presented an introduction and overview of the proposed methods and approaches. She explained the public health importance of ocular safety testing and hazard labeling and said that 15% of all eye injuries are due to chemicals. The Draize Rabbit Eye Test, which involves instillation of 100 µL (liquids) or 100 mg (solids) of a

test substance into the lower conjunctival sac of one eye of a rabbit, is the *in vivo* test method currently accepted by US Federal and international regulatory agencies.

The Ocular Peer Review Panel, “the Panel,” met on May 19 -21, 2009; their report will be available in July. ICCVAM plans to transmit recommendations to Federal agencies in December and request responses by June 2010.

The Panel evaluated:

- Routine use of topical anesthetics, systemic analgesics, and humane endpoints to avoid or minimize pain and distress during *in vivo* ocular irritation testing
- Validation status of four *in vitro* test methods for identifying mild/moderate ocular irritants and substances not labeled as irritants: BCOP, ICE, HET-CAM, and IRE
- Validation status of the *in vivo* low volume eye test (LVET)
- Validation status of the individual test methods and testing strategies to assess eye irritation potential of AMCPs, including use of the BCOP, Cytosensor Microphysiometer® (CM), and EpiOcular™ (EO) test methods

Dr. Merrill briefly reviewed the procedures for conducting the test methods, summarized the test method data, and then presented ICCVAM’s draft proposed recommendations for their use and limitations. She then summarized the ICCVAM charges to the Panel and acknowledged ICCVAM and the ICCVAM Ocular Toxicity Working Group.

Dr. A. Wallace Hayes, Harvard School of Public Health and Peer Panel Chair, presented a summary of the Panel report. The Panel was composed of 22 members from six different countries and they came to complete consensus on all but one of the recommendations (see HET-CAM below). He acknowledged the support of NICEATM and in particular, the contract support staff. He detailed the ICCVAM charges to the Panel and summarized the Panel’s recommendations:

- The Panel proposed an alternative preemptive pain management protocol that should be used for all *in vivo* rabbit eye irritation tests intended for regulatory safety testing, unless there is requirement for monitoring the pain response.
- The Panel concluded that, based on the available data and information, some humane endpoints recommended by ICCVAM are adequate to terminate a study.
- The Panel supported the ICCVAM draft recommendation that the available data and ICE test method performance do not support its use to identify substances from all hazard categories as defined by Globally Harmonized System (GHS), EPA, and EU classification systems.
- The Panel agreed with the ICCVAM draft recommendation that the available data and ICE test method performance do not support its use as a screening test to identify substances not labeled as irritants from all other hazard categories as defined by GHS, EPA, and EU classification systems.

- The Panel supported the ICCVAM draft recommendation that the available data and BCOP test method performance do not support its use to identify substances from all hazard categories as defined by GHS, EPA, and EU classification systems.
- The Panel agreed with the ICCVAM draft recommendation that the available data and BCOP test method performance support its use as a screening test to identify substances not labeled as irritants when results are used for EU or GHS hazard classifications.
- The Panel concluded that the BCOP test method cannot be used as a screening test to identify EPA Category IV substances.
- The Panel supported the ICCVAM draft recommendation that the available data and HET-CAM test method performance do not support its use to identify substances from all hazard categories as defined by GHS, EPA, and EU classification systems.
- The Panel (with one minority opinion) did not support the ICCVAM draft recommendation that the available data and HET-CAM test method performance support its use as a screening test to identify substances not labeled as irritants when results are used for EU or GHS hazard classifications.
- The Panel concluded that additional optimization and validation studies that include all four recommended endpoints are needed before definitive recommendations on the relevance and reliability of the IRE test method can be made.
- The Panel concluded that in the absence of all data, including the ECVAM BRD, they could not make definitive conclusions or recommendations on the validation status of the LVET.
- The Panel agreed with the ICCVAM draft recommendation that the CM test method can be used as a screening test to identify both ocular corrosive/severe irritants and substances not labeled as irritants in a tiered-testing strategy, as part of a weight-of-evidence approach, but this use is limited to surfactant chemicals and specific types of surfactant-containing formulations (e.g., cosmetics and personal care products).
- The Panel agreed with the ICCVAM draft recommendation that there were insufficient data to support use of the AMCPs testing strategy (i.e., using the BCOP, CM, and EO test methods) for classification of substances in all four ocular hazard categories.
- The Panel agreed with the ICCVAM draft recommendation that there were insufficient available data on which to base definitive recommendations on the proposed alternate testing strategy (i.e., using the BCOP and EpiOcular™ test methods) for classifying substances in all four ocular hazard categories.
- The Panel recognized that the use of histopathological evaluation as an additional endpoint does not improve the accuracy and predictability of the BCOP test method for the limited database of currently tested AMCPs; however, histopathological evaluation may prove to be a useful endpoint and as such, collection of ocular tissue and further efforts to optimize histopathological evaluation is strongly encouraged.

Dr. Levine said she saw nothing in the flow chart that required all three tests to be used at the same time. Dr. Hayes said that the concern of the Panel was that it would have been very helpful to know comparative results of compounds tested in all three tests to allow them to adequately evaluate the overall performance of the proposed testing strategy.

### **B. Public Comments**

Dr. Rodger Curren, IIVS, asked the attendees to read the written comments he would be sending for posting on the Website. He addressed Dr. Levine's comment regarding materials not being tested in all three assays and said many antimicrobials were tested in each of the assay systems. Twenty-eight materials were fully evaluated in all the tests and there were no differences in results among the tests. He could understand if there were considerable differences in the chemistry of the materials, then testing in all three assays might be needed, but otherwise it was not. He suggested a way to strengthen the peer review process for additional studies going forward and to improve the efficiency of the reviews. He said there was no effective way for the proponents of an assay strategy or the developers of a new assay to interact with the Panel. Many questions arose in this and other reviews that could have been answered quickly by the writers of the BRD or the developers of the assay. The proponents of the assay were allowed to speak only to the methodologies and not to the interpretation. He was not proposing extended debate in the peer review process, but only some way to allow greater interaction with the Panel.

Dr. Kate Willett, PETA, expressed puzzlement that the Panel's evaluation involved such an enormous review when the original nomination was simply for the antimicrobial project.

Dr. Levine asked if EPA's specific charge to ICCVAM, regarding a review of the flow chart's use for making labeling decisions on AMCPs, was communicated to the Panel. Dr. Stokes said the charge was clearly communicated. He further added that bringing a peer review Panel together is very expensive and time-consuming process; therefore, NICEATM-ICCVAM wanted to take advantage of convening this international Panel of experts by having other related test methods reviewed. NICEATM-ICCVAM had other topics they wanted to review, so they consolidated them for one Panel at one meeting. It resulted in an aggressive agenda and the Panel was very thorough. They took their time to do a careful, comprehensive review that in the long-term would benefit the entire project.

### **C. SACATM Discussion**

Dr. Freeman asked Dr. Levine about EPA's notice of proposed rule making for GHS adoption and whether EPA would adopt the GHS classification system. Dr. Levine said no decision would be made until a new Assistant Administrator is confirmed. Dr. Freeman said the Classification, Labeling and Packaging regulation in the EU system, which represents their acceptance of GHS, has been released. The EU system will merge with GHS in 2010. He said the GHS system represents the future and he was unsure what the United States is doing regarding the three scoring methods. He expressed confusion regarding the earlier conclusion for the use of BCOP to screen for



corrosives or severe irritants and the newer conclusion for its use to screen for substances not labeled as irritants using the GHS or EU system. He said it was at opposite ends of the spectrum and that if the United States were going to adopt GHS in the future, the EPA method should not matter. He expressed concern about classification of materials between those identified as severe irritants and non-irritants. Dr. Levine compared this classification to the issue with classifying skin irritation, where identification of the extremes is possible. She considered it a learning opportunity and suggested other agencies should also address this issue.

Dr. Barile, a lead discussant, agreed with Dr. Freeman. He understood from the 2006 review that both BCOP and ICE were approved for identifying corrosives and severe irritants, but in the 2009 conclusions, only BCOP was approved for the classification of corrosives and severe irritants. Dr. Stokes clarified that BCOP and ICE are still recommended for identifying corrosives and severe irritants. In the 2009 review, the recommendations for the use of ICE have not changed; ICE was not recommended for the identification of all ocular hazard categories as defined by the EPA, EU, and GHS classification systems. In addition, ICE was not recommended as a screening test to identify substances not labeled as irritants from all other hazard categories as defined by the GHS, EPA, and EU classification systems. Dr. Stokes emphasized that one of the reasons NICEATM-ICCVAM is using the term "not labeled as irritant," is that under the EU and GHS classification systems, even if a material is considered "not labeled as an irritant," it can still cause a considerable amount of irritation. For example, 43 substances not classified as irritants in the GHS or EU scheme are EPA Category III or higher. Category III substances cause lesions that persist for more than 24 hours, but clear by seven days. Dr. Stokes also noted that the IRE was not recommended because there are not enough data using all four endpoints, as in the current ICCVAM-recommended protocol. HET-CAM was proposed by ICCVAM to identify non-labeled surfactants and surfactant-containing compounds. The Panel disagreed with the ICCVAM recommendations because they considered the number of substances in the intermediate irritancy categories (i.e., mild and/or moderate irritants) to be insufficient.

Dr. Barile asked about use of the CM in ocular testing and the status of the testing, given that the machine is no longer available. Dr. Stokes said a new version of the CM is being developed that will measure additional endpoints. The new machine will need to meet or exceed the performance for the existing CM. Dr. Barile said little information had been presented on the CM as to what it tested and he asked why mouse fibroblasts were used. He suggested a more extensive review of CM by ICCVAM and more background information. Dr. Levine said the EPA has a policy of not recommending a brand or product; guidelines are based on performance standards. Dr. Barile also asked about use of the 2006 BRD database. Dr. Merrill said data from the AMCP submission were added to the BCOP database from 2006, but the available database for ICE had not changed since 2006.

Dr. Fox, a lead discussant, said he agreed with the report but had some comments on the science. The CM is an antiquated tool that is not sophisticated enough for use in ocular methods; there are better tools available. The methodology should be validated, not the instrument. He said in the original review, the BCOP was found acceptable to

detect corrosives, but has been upgraded to detect non-labeled materials. Dr. Stokes said more data had been added from the AMCP submission. BCOP was originally evaluated for its accuracy in classifying substances as either severe or non-severe, with irreversible or reversible effects, respectively. Accuracy for identifying moderate, mild, and non-labeled categories was not performed in the original review. Dr. Freeman said there was some dissension on the BCOP conclusions in 2006. Dr. Fox said that the local anesthetics recommended for use are esters, which have short half-lives; he asked why amides, which are longer acting, were not chosen for use. A disadvantage of local anesthetics is that they create tear breakup time and allow the compound increased access to the eye. He said a topical ophthalmic amide anesthetic might be a better option for pain control in the Draize test.

Dr. Karen Brown, a lead discussant, said the use of anesthetics for the Draize test was overdue. She said it should be a requirement unless there is justification for non-use. Systemic anesthesia should be used as well as topical anesthetics. She agreed with the Panel's recommendations, but asked for more information on the two AMCP testing strategies saying more work should be done in that area and it should move forward quickly. Individual tests were done with the BCOP and EO and it appeared they could differentiate severe from moderate and mild AMCPs. She asked how companies could be encouraged to generate more data for the AMCPs, similar to GlaxoSmithKline doing more research on the IRE. Dr. Stokes said the Ocular Toxicology WG's recommendation was to encourage industry to generate more data. Accordingly, the EPA just issued a proposal for a pilot project to encourage industry to generate data that would utilize the methods in the strategy. Dr. Levine said the EPA is proposing an eighteen-month pilot. Companies will provide both *in vitro* data and Draize data on similar products. The project will collect incident information on products that have been on the market for eight to ten years without labeling. The EPA will then make labeling decisions and evaluate how it is working. Dr. Karen Brown said the sequence of tests looked very promising.

Dr. Hansen, a lead discussant, concurred with the previous comments and said it was encouraging and long overdue that ICCVAM was moving toward requiring topical anesthetics and systemic analgesics.

Mr. Wnorowski, a lead discussant, said his company had developed some of the data several years ago on the anesthetics. His company has been successfully using anesthetic pretreatments for all its studies. He supported the other models moving forward and being accepted for regulatory purposes.

Dr. Freeman concurred with discussion on the use of anesthetics in the Draize test. Dr. Meyer asked how much is enough with respect to ICCVAM, and would the regulatory agencies accept a partial solution in the identification of classes II, III, and IV. Most of the pain and distress occurs with class I chemicals. She suggested moving forward rather than continuing to address the low rates of performance for the other classifications. Dr. Ehrich asked Dr. Hayes about the Panel's specific recommendation for the use of the analgesic buprenorphine. Dr. Hayes said this was based on strong recommendations from the veterinary anesthesiologist and ophthalmologists on the

Panel, based on their clinical experience. He said the important concept was to use a systemic analgesic first followed by a topical anesthetic prior to test substance application, and then to continue treatment with systemic analgesics as long as necessary.

Dr. Charles said harmonization is needed for assessing the performance criteria for the assays from a drug development perspective. Once there is harmonization, there is a need for guidance and strategy. He suggested assessing the other methods in a similar fashion to the AMCPs, and categorizing the test article based on a multiple assay strategy as opposed to doing more work on each individual assay. Dr. Stokes said an ECVAM-sponsored workshop suggested a top-down, bottom-up approach using a three-category system. An *in vitro* test or battery of tests would be needed that could identify all substances that could cause irreversible effects (i.e., all category I substances, with a high degree of certainty). All other categories would involve reversible damage and not cause permanent effects. Another test or battery would then be used only to identify substances that do not cause significant irritation (i.e., non-labeled substances). It would not require a high degree of sensitivity, but would identify most substances in this category without significant over-labeling. All other substances would be classified as mild or moderate. Further testing could be done to differentiate mild and moderate substances yielding a lower hazard warning for mild substances. This top-down, bottom-up approach is being pursued for both dermal and ocular irritation. Dr. Stokes explained that for ocular testing the methods are not available for the top or bottom for the level of performance needed. Not enough data are currently available to support a completely non-animal approach. Dr. Freeman said it was debatable because the BCOP could identify the highs and lows using GHS. Dr. Stokes said there are significant restrictions on categories of substances for which BCOP can be used, as some chemical classes and physical properties result in significant false negative results, which would not be acceptable in a top-down decision model.

Dr. Nicolaysen asked why the Panel recommended the dose of 0.01 mg/kg buprenorphine, which is lower than the 0.05 mg/kg used clinically. Dr. Hayes said the dose was based on clinical experience. Dr. Nicolaysen said there should be better evidence to use the lower dose. Dr. Marilyn Brown expressed some concern about the handling-stress induced in animals with the administration of the both analgesics and anesthetics, but with differing dosing schedules. Dr. Corcoran said there did not appear to be a consensus standard of care. He thought the recommendations to be overly proscriptive and suggested establishing an expectation for care with the goal of relieving pain with an antinociceptive and an anesthetic at appropriate doses and dosage schedules.

### **June 26, 2009**

Dr. Freeman reconvened the meeting at 8:30 A.M. Attendees introduced themselves and Dr. White read the conflict of interest statement.

## **XI. Report on the Second Meeting of the Independent Peer Review Panel: Evaluation of the Updated Validation Status of New Versions and Applications of the Murine Local Lymph Node Assay (LLNA)**

### **A. Presentations**

Dr. Paul Brown, FDA and member of the Immunotoxicity WG, provided an introduction and overview of the proposed LLNA methods and applications. He said the traditional LLNA was reviewed by ICCVAM in 1998 and again in 2008. He outlined some of the regulatory requirements for skin sensitization evaluation that currently exist and then provided an overview of the LLNA test method protocol. The purpose of the LLNA is to identify chemical sensitizers through quantification of lymphocyte proliferation. A Stimulation Index (SI) is calculated as the ratio of radioactivity incorporated into draining auricular lymph nodes cells of treated animals to that of vehicle control animals. In 2008, the peer review panel agreed with ICCVAM that more data were needed to evaluate three modified versions of the LLNA not requiring radiolabeling and application of the LLNA for pesticide formulations, other products, and substances tested in aqueous solutions. Additional data were submitted to NICEATM and ICCVAM. The Immunotoxicity Working Group (IWG) working with NICEATM revised the draft BRDs, and ICCVAM updated the draft test method recommendations.

Dr. Paul Brown provided overviews of the protocols, some details of the test method data, and a summary of the draft ICCVAM recommendations:

- The LLNA: Daicel Adenosine Triphosphate (DA) test method with specific, defined limitations can be used to identify substances as potential skin sensitizers and nonsensitizers.
- Substances that produce  $SI > 1.7$  and  $< 2.5$  should be evaluated using an integrated decision strategy with all available and relevant information.
- The LLNA: Bromodeoxyuridine enzyme-linked immunosorbent assay (BrdU-ELISA) test method with specific, defined limitations can be used to identify substances as potential skin sensitizers and nonsensitizers. Substances that produced  $1.3 \leq SI < 2.0$  should be evaluated using an integrated decision strategy with all available and relevant information.
- The LLNA: BrdU Flow Cytometry (FC) test method appears useful for identifying substances as potential skin sensitizers or nonsensitizers; however, more information and data are needed before ICCVAM can make a recommendation.

Regarding the applicability domain of the LLNA, Dr. Paul Brown said ICCVAM had comprehensively updated data and information on 104 pesticide formulations, 6 textile dyes, 12 natural complex substances, and 24 substances tested in aqueous solutions. Based on these data, ICCVAM had the following draft recommendations:

- The LLNA is more likely than a guinea pig test to classify a pesticide formulation as a sensitizer.
- More data are needed before a recommendation on the use of the LLNA for testing dyes can be made.
- A definitive recommendation on the use of the LLNA for testing natural, complex substances cannot be made until a larger number of known human sensitizers have been tested.

- LLNA is more likely than a guinea pig test to classify a substance tested in an aqueous solution as a sensitizer. LLNA has utility for hazard classification of substances tested in aqueous solutions provided that the potential for possible over-classification is not a limitation.

Dr. Paul Brown said the ICCVAM Independent Scientific LLNA Peer Review Panel meeting was held April 28-29, 2009, in Bethesda, MD. The panel consisted of 15 experts from six countries.

Dr. Diggs asked about the negative aspects of over-regulation. Dr. Paul Brown said it would depend on the agency. At the Center for Drug Evaluation and Research, where drugs that will be used intentionally for benefit in humans are regulated, over-classification can have negative effects. Dr. Levine said the EPA tries not to over-label because it would dilute the utility of the labeling; people would stop paying attention to the labels. Dr. Freeman said over-classification could have a commercial impact and possibly lead to product deselection when the product has real value.

Dr. Michael Luster, West Virginia University, chaired the panel and provided highlights of the panel report. He thanked the panelists, the Evaluation Group Chairs, Drs. Michael Olson, Stephen Ullrich, and Michael Woolhiser, and the NICEATM staff. He reviewed the ICCVAM charges to the Panel and the modifications and applications to be reviewed.

Dr. Luster then presented the abbreviated highlights of the Panel's report:

- LLNA: DA - The available data and test method performance support its use to identify substances as potential skin sensitizers and nonsensitizers, with certain limitations. Based on the current validation database, multiple SI values should be used as decision criteria to identify sensitizers and nonsensitizers.
- LLNA: BrdU-ELISA - The available data and test method performance support its use to identify substances as potential skin sensitizers and nonsensitizers, with certain limitations. Based on the current validation database, multiple SI values should be used as decision criteria to identify sensitizers and nonsensitizers.
- LLNA-BrdU-FC - The database of more than 45 representative test substances yielded adequate accuracy based on results from one laboratory; intralaboratory reproducibility had also been adequately demonstrated; however, a recommendation on the validity of this test should be deferred pending an independent audit of the data and an interlaboratory validation study, both of which the Panel recommended. If both of these issues can be successfully addressed, then the assay should be considered scientifically validated as an alternative method for the traditional LLNA.
- All three of the nonradiolabeled LLNA protocols are mechanistically and functionally similar to the traditional LLNA and therefore, do not require separate test method performance standards.
- An emphasis should be made to include ear swelling measurements and/or immunophenotypic markers as an indicator of irritation for the traditional LLNA and for any modified LLNA test methods.

- Any material should be a candidate for testing in the LLNA unless there are unique physicochemical properties associated with the class of test materials that might affect its ability to interact with the normal immune processes. Therefore, the LLNA should be considered applicable to pesticide formulations, other products, and substances in aqueous solutions unless there is a biologically based rationale for exclusion.
- The Panel expressed a strong desire to avoid revalidation of the LLNA for new classes/types of test substances unless there is a biologically based rationale. If any variant of the LLNA is validated for use to test novel classes, then the findings should be relevant to the family of validated LLNA tests.

Dr. Freeman asked about using the lower cut-off values as the thresholds for positive or negative labeling, in order to make decision-making more straightforward. Dr. Luster said it was a small database, the error rate for positives was too high, and it might cause misuse of the methodology. If LLNA results are indeterminate, a guinea pig test may need to be done, but overall, fewer guinea pigs will be used and the end result will benefit animal welfare. The Panel discussed peptide reactivity as a good predictor of the LLNA, but did not make a recommendation on it. Dr. Fox expressed concern for using the adenosine triphosphate (ATP) assay, deeming it a poor assay for measuring proliferation. He questioned the BrdU methodology and suggested some alternatives. Dr. Luster said the Panel did not formally discuss ways to improve the assays.

Dr. Fox said FC is the most sensitive and promising assay and Dr. Luster agreed. Dr. Freeman asked about the cost of the LLNA using FC compared to the other assays. Dr. Luster said costs include the instrument and trained personnel. He said immunophenotyping was used separately to identify irritants from sensitizers, but was not part of the Panel's review. Dr. Freeman asked about accuracy and sensitivity of the FC compared to humans or guinea pigs. Dr. Luster said the results equivocated somewhat, but that only a few chemicals did not show the same results. Dr. Fox said a two-channel fluorescence-activated cell-sorting machine is cheaper and easier to calibrate than a scintillation counter. Dr. Luster agreed due to the cost of disposal of <sup>3</sup>H-thymidine. Dr. Marilyn Brown said it is essential to assess the LLNA in relation to human data when available, and asked about the actual use of LLNA compared to guinea pig tests. Dr. Levine said the EPA is getting a fair number of LLNAs now, which should increase when companies know it is accepted. Dr. Meyer asked about statistical expertise on the panel and about comparing continuous and percentage data. Dr. Luster said there were two statisticians and they did not discuss that issue.

Dr. Hansen asked about tracking the frequency of submissions, acceptances, and revisions by registrants. Dr. Levine said the EPA does not track submissions, but has done rejection analyses on particular studies. She will suggest tracking at EPA. Dr. Luster said the OECD might have tracking information because the original LLNAs, for which they have a large database, were developed in Europe. Dr. Freeman was unsure about the outcome of recommendations once the agencies received them, so it would be good to have such information from agencies made publicly available; it may encourage further use of the methods. Dr. Fitzpatrick asked if drug sponsors might be willing to share that information with ICCVAM. Dr. Paul Brown said the FDA does not

formally track submissions, but a number of LLNA assays have been submitted. In FDA's pre-meeting discussions, sponsors were told that the LLNA is acceptable. Assays have not been rejected unless there is a problem with the particular assay. Dr. Levine asked about mixtures that contain a small component of sensitizing material, creating the possibility of false negatives, and the potential for the interaction of components in a mixture to be a sensitizer when the individual components are not. Dr. Luster said the approach is to test the individual material, the vehicle, and the mixture separately. There are examples of interaction in mixtures that have the potential to destroy the epidermis, so it is important to test the combination. Dr. Levine asked about waiving testing on new formulations if they are fairly similar to existing formulations. Dr. Luster said it would be up to the regulatory agencies, but cautioned that formulations can change between batches and between companies. Dr. Levine suggested more limited testing on pesticide formulations, which are produced in series that vary only in active ingredients. Dr. Charles asked about the use of sodium lauryl sulfate (a potential sensitizer) pretreatment in the DA assay. Dr. Luster said the data had not been obtained, but the Panel did not think it would change the outcome of the recommendation.

#### **B. SACATM discussion**

Dr. Freeman asked whether SACATM could provide advice about the priority for the inter-laboratory validation studies for the FC assay. Dr. Stokes said ICCVAM accepts nomination for evaluation or validation of test methods and then decides a draft priority, which is presented to SACATM. In this case, SACATM is presented a proposed activity, which it discusses, decides on a priority, and makes a recommendation to ICCVAM.

Regarding false positives, Mr. Wnorowski, a lead discussant, asked if the next step would be guinea pig testing and which test would carry more weight. Dr. Levine said from regulatory point of view, the most conservative tests would be used. If the weight of evidence includes human data and there is a potential for over-prediction by the LLNA, then that would be taken into consideration in the labeling. Dr. Freeman suggested using guinea pig studies for those substances in the indeterminate range. Dr. Levine said a line is included on pesticides stating, "the product may cause allergic reaction in sensitive individuals." Companies developing consumer products may abandon them if they are deemed sensitizers, so the company must make the decision about what testing is done.

Dr. Meyer, a lead discussant, expressed concern regarding the comparison of different statistical analyses between the FC and ELISA methodologies and felt this issue should be addressed. Dr. Stokes responded that before the BRD is finalized, ICCVAM would consult with a statistician to make sure the appropriate analyses were done. Dr. Meyer asked about the behavior of different classes of compounds in different assays, especially the aqueous substances, which should not go through the stratum corneum. She asked if the sodium dodecyl sulfate pre-treatment for permeability had ever been validated. Dr. Luster said it was not included in the data from the sponsors. Dr. Meyer said such treatment might explain why the different classes of compounds performed so

differently in the tests. She asked to see the statistics on the FC test before making a recommendation.

Dr. Ehrich, a lead discussant, expressed strong support for the Panel's report. She said the LLNA DA method looked ready for release. The submitter had done the appropriate steps to meet the recommendations of the 2008 panel. She said the assay is not easy technically, which is why variability is an issue. Inter- and intra-laboratory studies have been done and she supported the Panel's conclusions. The LLNA DA is more sensitive than the ELISA test method, but the intermediate range for both test methods needs to be further defined and reevaluated. No new data were presented for the ELISA beyond the 2008 panel report. Dr. Ehrich supported giving high priority to the FC inter-laboratory studies and agreed that there should not be separate performance standards for the non-radioactive methods. Some intermediate areas still exist, but could be handled on a case-by-case basis. Additional performance standards would only add unnecessary delay to the release. She said it is important to provide non-radioactive tests, since some places do not allow radiation. Testing for mixtures, pesticide formulations, aqueous solutions, and metals is improved since the 2008 report. There are still some substances that are difficult to test, but there is no reason to continue to use radiolabeled testing in guinea pigs.

Dr. Charles, a lead discussant, generally concurred with Panel's recommendations and agreed giving a high priority to the FC testing. The use of dual ranges in the DA and ELISA assay for assessing sensitizers versus non-sensitizers could potentially place many compounds in limbo, so the decision criteria should be reassessed as more data are obtained. He concurred with the suggestion to include evaluation of ear swelling as an indicator of irritation and immunophenotypic marker assessment. The BRD formulations tested included many potential false negatives relative to the guinea pig maximization test (GPMT). He agreed that the GPMT was never fully validated for formulations and possibly under-predicts relative to the LLNA.

Dr. Barile, a lead discussant, suggested including data on accuracy, specificity, sensitivity, and performance standards that were available only in the BRDs from last year. He found it hard to make suggestions on applicability since new substances were added to the test formulations without including the performance data. He approved of the two decision criteria to allow specific cut-off points. He questioned the concern about the lack of human data, which are hard to obtain, and why comparisons with animal data are not enough. He questioned the prohibitions on using radioactivity in other countries and stated that radioactive procedures are very sensitive, though costly, and should not be discarded. He asked about the development of non-animal tests for detecting sensitizers. Dr. Stokes mentioned the human Cell Line Activation Test (h-CLAT) method undergoing validation in Japan and the peptide reactivity assays submitted for validation by Proctor and Gamble. Because of the Cosmetics Directive in Europe, which will completely ban the use of animals for repeat dose studies by 2013, there is much interest in developing non-animal methods to assess allergic contact dermatitis. Dr. Barile said he would like to see more discussion regarding the biology and mechanisms that are the bases of the tests, such as what is being tested by the LLNA, what cell types are proliferating, and which mouse strain is being used. He



suggested making the non-animal testing a priority over the FC tests. Dr. Fox suggested the compounds be tested for photoactivation and photosensitization. He agreed with Dr. Barile that non-animals methods should have the highest priority. Dr. Stokes clarified that ECVAM has the lead on three non-animal validation studies, which are a high priority in Europe. Dr. Kreysa added that ECVAM had received three submissions for non-animal test methods for skin sensitization and are planning validation studies now. Using these three test methods in a testing strategy could possibly serve as a replacement for animal tests.

Dr. Paul Brown said the FDA typically does not do non-clinical testing of drug products for photoallergenicity. Topical products are usually tested in a human photoallergenicity study and a repeat patch test for allergenicity in humans. Those results determine further clinical development and assessment for hypersensitivity reactions; therefore, the FDA would eventually get definitive human data to characterize photosensitivity of a product. Dr. Fox encouraged testing for photoallergenicity and said the assay does not address it. Dr. Luster said there are LLNA data on photosensitization. Dr. Meyer encouraged the development of non-radioactive methods, which are easier to teach, and said ELISAs are easier technically to teach than FC. Dr. Corcoran asked about thresholds and the boundary between positive and non-positive responses in the LLNA and the guinea pig test. Dr. Luster said false positives were an issue with pesticide formulations. In the old GPMT, the substance was just put on the skin. Now, 1% pluronic acid can be used as detergent to increase dermal penetration of water-soluble substances. Mr. Wnorowski said the GPMT is generally considered more conservative and more likely to give false positives than the Buehler test; whereas the Buehler test tends to give a positive response less often. The sensitivity of the human test is intermediate. The LLNA is the most conservative and generates the most false positives. Many registrants consider that unacceptable and would be reluctant to label the product as a sensitizer. Dr. Corcoran hoped to hear that the LLNA identified sub-positive responses, creating a weight of evidence argument against labeling. He thought the LLNA's rate of false positives caused over-classification and could be a disincentive for its use. Mr. Wnorowski concurred. Dr. Levine said from a regulatory perspective, it is possible to eliminate the Buehler test if replaced by another test. Dr. Freeman, a member of the original LLNA review panel, did not recall that the LLNA over-predicts compared to the GPMT. He suggested for complete transparency that the final report should reflect the performance of the various tests. Dr. Stokes said ICCVAM would extract those data from the 1999 TMER. ICCVAM has done all the analyses, and the overall accuracy of ~70% was comparable to the predictivity of the LLNA for existing human data and the combined Buehler-GPMT tests for human data. The overall accuracy of the LLNA for predicting the GPMT was about 88%. The difference of 15 % could be due to over-prediction compared to the GPMT.

Mr. Wnorowski expressed concern about the limited, additional data for the pesticide formulations. Compared to the original assays on pure chemicals, these data show that the pesticide formulations appear to produce false positives in the LLNA compared to the guinea pig-based tests. Dr. Allen clarified the difference in sensitivity between the Buehler test and the GPMT. For the 22 substances for which there were comparative tests, 20 of the guinea pig tests were actually Buehler tests, so there is a question as to

whether they could have been concordant if they had been GPMTs. Strictly comparing the performance of the LLNA and the GPMT for those 22 substances, the accuracy is not great because the trend was to get a positive result more often in the LLNA. The original concern about the use of LLNA for mixtures was that the LLNA would give false negatives, but it is actually more conservative. Mr. Wnorowski agreed and expressed concern that if the LLNA is too conservative, it will not be used unless regulatory agencies require it, because of its impact on the marketing of products.

Dr. Marilyn Brown said laboratories have moved away from using the LLNA because it is the only test that uses radioactivity. Providing a LLNA test that doesn't use radioactivity would increase its use.

Dr. Freeman asked for a vote on whether NICEATM-ICCVAM should set a high priority on the inter-laboratory validation of the FC method because the only currently data are from just one laboratory. Dr. Corcoran said everything cannot be high priority and that doing the FC validation would mean that ICCVAM could not do something else. Dr. Stokes agreed and said the vote would be advice for the NTP and NICEATM to make decisions about competing priorities for limited resources. SACATM has not provided advice on nominations for validation studies for two years, and ICCVAM currently has no new nominations for validation studies. Dr. Diggs seconded the motion. SACATM voted 9 yes, 1 no (Dr. Meyer), 1 abstention (Dr. Barile), and 1 recusal (Dr. Marsman). Dr. Meyer voted against the motion because she was uncomfortable with the statistics and thought the ELISA is a better method to move forward. Dr. Barile abstained because he thought the other two tests should have equal priority and because FC is difficult to use for training and is costly. Dr. Fox suggested lowering the priority of the ATP assay because it is technically flawed. Dr. Stokes said all SACATM comments would be considered in finalizing the recommendations of the IWG and ICCVAM.

## **XII. Updates on JaCVAM and ECVAM**

### **A. JaCVAM Update**

Dr. Kojima provided an update on the activities of JaCVAM. Japan was one of the four countries that entered into the ICATM agreement. Dr. Kojima attended as a representative of Dr. Masahiro Nishijima, who signed the MOC on behalf of Japan. He described a test guideline for a Stably Transfected Transcriptional Activation (STTA) Assay for the detection of estrogenic (agonist) activity of chemicals that was accepted by OECD in April 2009. Other projects include: a new test guideline for a STTA assay for detecting the anti-estrogenic activity of chemicals, a comet assay for genotoxicity testing, a cell transformation assay using the Balb/c 3T3 cell line, a new test guideline for an *In Vitro* Skin Irritation Assay (LabCyte model), and a non-radioisotope version of the LLNA.

He described the JaCVAM framework for peer review and regulatory acceptance of alternative methods. JaCVAM has a steering committee that has supported a validation management team and established an oversight committee. The oversight committee prepares the BRD, which is evaluated by a peer review panel. The panel publishes a report that goes to the Regulatory Acceptance Board, which is somewhat similar to

SACATM. JaCVAM receives a report from the Board and then prepares a statement for Japan's regulatory agencies. Dr. Kojima submitted two statements last year: (1) the VitroLife-Skin™, a 3-dimensional cultured skin model for skin corrosivity testing and (2) the LLNA-DA for skin sensitization testing. The methods accepted by the JaCVAM Regulatory Acceptance Board were BCOP, ICE, and a battery system to predict phototoxicity (the Yeast Growth Inhibition Phototoxicity Assay and Red Blood Cell Photohemolysis Assay). The LLNA:BrdU-ELISA and *in vitro* skin irritation assay (EPISKIN) are pending acceptance.

JaCVAM has several on-going peer reviews for immunotoxicity (rLLNA), skin irritation (EpiDerm, Skin Ethics), eye irritation (cytotoxicity and cell function based assays), pyrogenicity (five *in vitro* assays), and acute toxicity testing (3T3/NRU). Ongoing validation studies include h-CLAT, *in vivo/in vitro* Comet assay, STTA, LUMICELL® ER assay, and Bhras cell transformation assay. Dr. Kojima listed JaCVAM's oral and poster presentations at the 7<sup>th</sup> World Congress on Alternatives and Animal Use in the Life Sciences that will be presented later this summer. He provided some information on and screen shots of JaCVAM's new website that was launched in March.

Dr. Barile asked about the composition of JaCVAM's peer review panel and the Regulatory Acceptance Board. Dr. Kojima explained that different people comprise the two groups. Dr. Stokes acknowledged Dr. Kojima's hard work on JaCVAM and his service on many of the ICCVAM and ECVAM WGs.

## **B. ECVAM Update**

Dr. Kreysa updated SACATM on the activities of ECVAM. He explained that the European Commission Joint Research Centre Institute for Health and Consumer Protection has a new structure consisting of five units: Molecular Biology and Genetics; Nano-biosciences; *In Vitro* Methods; Systems Toxicology; and Chemical Assessment and Testing. ECVAM now has easier access to resources and will be interacting primarily with *In Vitro* Methods and Systems Toxicology, among other groups, to advance high and medium throughput methods and generate more data. The *In Vitro* Methods unit has four groups: Coordination of Method Validation, In-house Validation and Training, *In Vitro* Method Optimisation, and *In Vitro* Method Information Management.

He reviewed ECVAM's mission statement and explained that priorities are determined by EU legislation such as: Registration, Evaluation, Authorization, and Restriction of Chemical substances (REACH); cosmetics (replacement of animal testing by 2013); pesticides (alternative methods for endocrine disruptor testing by 2013); and animal protection and welfare. Some challenges to be faced are that complex endpoints require complex test methods, complex test methods require complex validation, and complex test methods must be applied and accepted. There is an increasing demand for alternative methods created by the EU legislation and globally increased interest. To handle the challenges and workload necessitates transparent decision-making during the validation procedure, which involves: (1) test submission, (2) assessment if tests meet ECVAM criteria for entering pre-validation, (3) a decision whether a pre-validated test should go into formal validation, and (4) independent peer review of validation

studies. ECVAM/Institute for Health and Consumer Protection will work to stimulate and support test development and stimulate test submissions for official validation.

Dr. Kreysa provided updates on the status of alternative testing methods for eye irritation, genotoxicity, carcinogenicity, reproductive toxicology, biologicals, food, ecotoxicity, and systemic toxicity. He said new test submissions are forthcoming and will lead to validations in the areas of skin irritation, eye irritation, skin absorption, and reproductive toxicology.

To better disseminate information, ECVAM is expanding knowledge management and databanks. The existing database on alternative methods to animal experimentation (DB-ALM) has had increasing registration and updated content. INVITTOX protocols will soon have remote data entry and content updating. Complementary activities include the ECVAM Guide on Good Search Practices, development of on-line test submission, and portal development. The European Partnership for Advancing Alternatives has held discussions to create a “one-stop shop” for all 3Rs-related information. He said ECVAM supports integrated testing strategies by holding workshops, conducting in-house research, and participating in research projects. There are currently no conclusions regarding validation of these strategies. Long term challenges include validation of methods that cannot be compared to generally accepted “gold standards,” convincing risk assessors and risk managers to base their decisions on alternative methods that cannot be compared to a “gold standard,” and getting alternatives more quantitative for providing the information needed for risk assessment.

Dr. Marilyn Brown asked about using electronic communication strategies for the workshops to allow global participation. Dr. Kreysa said ECVAM has videoconference capabilities that could potentially be used.

Dr. Karen Brown asked about confidentiality agreements with industry and the use of coded data by the validation management organizations without exposing confidential business information. Dr. Kreysa said ECVAM would have agreements and also confidentially declarations for ESAC and its peer review panel members. ECVAM has begun to address material transfer agreements with industry to obtain test substances and is working with eight global pharmaceutical industries to exchange information on test systems. Dr. Barile asked about ECVAM stimulating funding for research and development in the national laboratories and asked how ICCVAM might become more involved in funding. Dr. Kreysa said ECVAM is doing some in-house research and participates in larger research project; however, that participation is consuming significant manpower. Participation in this research allows ECVAM to guide the scientists so their data are more usable for pre-validation. ECVAM gets requests to join consortia to do research but is not funding research. National centers for alternative methods might be created in the EU to stimulate research. Dr. Meyer asked about funding for repeated dose toxicity testing, for which the cosmetics industry and the European Commission are jointly funding a 50 million Euro program. In addition, the cosmetics industry is providing other funding to help develop alternative methods for cosmetics testing by 2013.

### **XIII. Other Business**

Dr. Freeman said a summary of the minutes would be provided shortly. Dr. White announced the next meeting on June 17 – 18, 2010, at the EPA in Research Triangle Park, NC. Dr. Meyer asked about ICCVAM reporting to Congress and ICCVAM funding. Dr. Stokes said ICCVAM under the ICCVAM Authorization Act is required to publish a biennial report that describes its progress and activities. The next report will cover 2008-2009 and be available in spring 2010. He explained that while NICEATM and ICCVAM and their mandates are established under law as part of NIEHS, separate funding is not provided for ICCVAM or NICEATM.

Dr. Stokes closed the meeting by thanking SACATM noting the very constructive and useful comments and suggestions that had been provided. ICCVAM representatives and WG members are looking forward to addressing the suggestions as they move forward to finalizing the recommendations on the test methods just reviewed. ICCVAM will consider the advice provided on the Plan and updates will be provided at the next meeting. Dr. Stokes thanked the ICCVAM representatives, WG members, NICEATM, and Dr. White and her staff for their efforts in preparing for the meeting. He thanked the public attendees for their comments, which were an important part of the meeting. Dr. Wind, ICCVAM chair, apologized for missing the first day and said she appreciated the hard work SACATM has done with all the information they were given. She said SACATM has done an incredible job and given ICCVAM much advice to consider.

Dr. Freeman adjourned the meeting at 12:15 PM.