

Minutes from the November 30, 2006 SACATM Meeting

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I. LOCATION OF BACKGROUND MATERIALS/PRESENTATIONS AND FREQUENTLY USED ABBREVIATIONS

Background materials and presentations for the SACATM meeting are available on the SACATM meeting web site (directly at <http://ntp.niehs.nih.gov/go/167> or <http://ntp.niehs.nih.gov/> see "Advisory Board & Committees).

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| 3 R's | Replacement, Reduction, Refinement |
| CPSC | Consumer Product Safety Commission |
| DOT | Department of Transportation |
| EPA | Environmental Protection Agency |
| FDA | Food and Drug Administration |
| ICCVAM | Interagency Coordinating Committee on the Validation of Alternative Methods |
| ILS | Integrated Laboratory Systems, Inc. |
| ECVAM | European Centre for the Validation of Alternative Methods |
| JaCVAM | Japanese Center for the Validation of Alternative Methods |
| 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 |
| NCI | National Cancer Institute |
| NIH | National Institutes of Health |
| OECD | Organisation for Economic Co-operation and Development |
| SACATM | Scientific Advisory Committee on Alternative Toxicological Methods |
| USDA | U.S. Department of Agriculture |

II. ATTENDANCE

SACATM met on November 30, 2006, at the NIEHS in Research Triangle Park, North Carolina 27709. The following individuals attended the meeting:

SACATM Members

Frank Barile, Ph.D. (St. John's University)
Richard Becker, Ph.D. (American Chemistry Council)
June Bradlaw, Ph.D. (International Foundation for Ethical Research)
Grantley Charles, Ph.D. (Allergan)
Mary Jane Cunningham, Ph.D. (Houston Advanced Research Center)
George DeGeorge, Ph.D. (MB Research Laboratories)
Michael Dong, Ph.D. (California Department of Pesticide Regulation)
Nancy Flournoy, Ph.D. (University of Missouri - Columbia)
Donald Fox, Ph.D. (University of Houston)
James Freeman, Ph.D. (ExxonMobil Biomedical Sciences, Inc.) *Chair*
Daniel Marsman, D.V.M., Ph.D. (Procter & Gamble)
Annie Qu, Ph.D. (Oregon State University)
Martin Stephens, Ph.D. (Humane Society of the U.S.)

Primary ICCVAM Ex Officio Members

George Cushmac, Ph.D. (DOT)
Karen Hamernick, Ph.D. (EPA)
Jodie-Kulpa-Eddy, D.V.M. (USDA)
Paul Nicolaysen, V.M.D. (NIOSH)
Alan Poland, M.D. (NCI)
Leonard Schechtman, Ph.D. (FDA)
William Stokes, R.A.D.M., D.V.M., D.A.C.L.A.M., (NIEHS)
Marilyn Wind, Ph.D. (CPSC)

Ad hoc and Liaison Representatives

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Marion Ehrich, Ph.D. (VA-MD Regional College of
Veterinary Medicine)
Roger McClellan, D.V.M. (Consultant)

Thomas Hartung, M.D., Ph.D. (ECVAM)
Hajime Kojima, Ph.D. (JaCVAM)

NIEHS/NTP Staff and NTP Contract Staff

David Allen, Ph.D. (ILS)
John Bucher, Ph.D., D.A.B.T. (NIEHS/NIH)
Jeffrey Charles, Ph.D., M.B.A., D.A.B.T. (ILS)
Raj Chhabra, Ph.D., D.A.B.T. (NIEHS/NIH)
Neepta Choksi, Ph.D. (ILS)
Frank Deal, M.S. (ILS)
Allen Dearry, Ph.D. (NIEHS/NIH)
Sally Fields (NIEHS/NIH)
Debbie McCarley (NIEHS/NIH)

Sheila Newton, Ph.D. (NIEHS/NIH)
Michael Paris (ILS)
Catherine Sprankel (NIEHS/NIH)
Judy Strickland, Ph.D., D.A.B.T. (ILS)
Kristina Thayer, Ph.D. (NIEHS/NIH)
Raymond Tice, Ph.D. (NIEHS/NIH)
Michael Waters, Ph.D. (NIEHS/NIH)
Samuel Wilson, M.D. (NIEHS/NIH)
Douglas Winters, M.S. (ILS)
Mary Wolfe, Ph.D. (NIEHS/NIH)

Public

Sara Amundson (Humane Society Legislative Fund)
George Clark, Ph.D. (Xenobiotic Detection Systems)
Noe Galvan, Ph.D. (Clorox)
John Gordon, Ph.D. (Xenobiotic Detection Systems)
Sue Leary (Alternatives Research & Development
Foundation)

Randy McClelland, Ph.D. (ADMET
Technologies)
Bobbie Peterson (RTI International)
Joseph Ruiz, Ph.D. (Vesta Therapeutics)
Catherine Willett, Ph.D. (People for the Ethical
Treatment of Animals)
Joanne Zurlo, Ph.D. (National Academy of
Sciences)

III. WELCOME, INTRODUCTIONS, AND RECOGNITION OF RETIRING MEMBERS

Dr. James Freeman served as the SACATM Chair and called the meeting to order at 9:30 a.m. Individuals in the room were asked to introduce themselves. Dr. Samuel Wilson, Deputy Director of the NTP and NIEHS, made opening remarks and distributed certificates of service for two SACATM members in attendance whose terms expire: Drs. Flournoy and Stephens. Drs. Allen Dearry, Interim Associate Director of the NTP, and Leonard Schechtman, ICCVAM Chair, also made welcoming comments. Dr. Kristina Thayer read the conflict of interest statement for SACATM.

IV. ICCVAM-NICEATM UPDATE

A. Presentation

Dr. William Stokes, NICEATM Director and ICCVAM Executive Director, provided an overview of ICCVAM-NICEATM activities since the December 12, 2005 SACATM meeting. Major topics covered by Dr. Stokes included:

- ICCVAM-NICEATM test method evaluation activities:
 - *Botulinum Toxin Testing*. A scientific workshop on “Alternative Methods to Refine, Reduce and Replace the Mouse LD₅₀ Assay for Botulinum Toxin Testing” co-sponsored by ICCVAM, NICEATM, and ECVAM was held on November 13-14, 2006, to review the state-of-the-science of alternative methods that may refine, reduce, and replace the use of mice for botulinum toxicity testing, and to identify priorities for research, development, and validation efforts that might advance the use of 3Rs methods and approaches for this purpose. A workshop report will be

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released in 2007. Information about the meeting is available at

<http://iccvam.niehs.nih.gov/methods/biolodocs/biolowkshp/wkshpinfo.htm>

- *Cytotoxicity Methods for Estimating Acute Oral Systemic Toxicity.* On May 23, 2006, ICCVAM held an international scientific peer review panel meeting to review the validation status of two cytotoxicity test methods proposed for estimating starting doses for acute oral systemic toxicity tests. A SACATM working group provided comments on the peer review panel report at an August 3, 2006 public teleconference (<http://ntp.niehs.nih.gov/go/8202>). On October 30, 2006, ICCVAM released the test method evaluation report and finalized background review document. The test method recommendations will be forwarded to U.S. Federal agencies in 2007. Recommendations and details of the evaluation process can be found at <http://iccvam.niehs.nih.gov/methods/invitro.htm>.
- *In Vitro Ocular Toxicity Test Methods for Identifying Corrosives and Severe Irritants.* In November 2006, ICCVAM released the test method evaluation report “*In Vitro Toxicity Test Methods for Identifying Severe Irritants.*” This report discussed the following four methods: (1) the bovine corneal opacity and permeability (BCOP) test, (2) the hen’s egg test – chorion allantoic membrane (HET-CAM), (3) the isolated rabbit eye (IRE), and (4) the isolated chicken eye (ICE). The finalized test methods recommendations will be forwarded to U.S. federal agencies in 2007. The final background review documents (one for each test method) were made publicly available in August, 2006. Test method recommendations and background materials are available at http://iccvam.niehs.nih.gov/methods/ocudocs/ocu_brd.htm.
- *ICCVAM Evaluation of In Vitro Skin Irritation Test Methods.* ECVAM skin irritation validation studies of EpiDerm™ and EPISKIN™ were completed in 2006. ICCVAM plans to evaluate the usefulness of these methods for U.S. hazard classification schemes used by the EPA, the Federal Hazardous Substances Act (FHSA), and the United Nations Globally Harmonized System of Classification and Labeling of Chemicals (GHS).
- *ICCVAM Test Method Evaluation: In Vitro Pyrogenicity Test Methods.* ECVAM proposed the following assays as replacements to the rabbit pyrogen test: (1) the human PBMC/IL-6 *in vitro* pyrogen test (PBMC/IL-6), (2) the human whole blood/IL-1 *in vitro* pyrogen test (WB/IL-1) (3) the human whole blood/IL-1 *in vitro* pyrogen test: application of cryopreserved human whole blood (cryo WB/IL-1), (4) the human whole blood/IL-6 *in vitro* pyrogen test (WB/IL-6), and (5) an alternative *in vitro* pyrogen test using the human monocytoid cell line MONO MAC-6 [MM6] (MM6/IL6). ICCVAM will hold a peer review panel meeting on February 6, 2007 to discuss the validation status of these assays. The panel report from that meeting is expected to be released by April and a final ICCVAM test method evaluation report issued in the fall of 2007.
- *Endocrine Disruptor Test Method Activities.* ICCVAM is addressing four endocrine-related activities:
 - The nomination of an MCF-7 cell proliferation assay to assess estrogenic activity (discussed below under “ICCVAM Nominations.”)
 - Providing comments on the draft OECD test guideline: “The uterotrophic bioassay in rodents: a short-term screening test for (anti)oestrogenic properties.”
 - Updating the list of reference substances recommended for *in vitro* endocrine disruptor validation studies. Six substances on the original list of 78 substances for both estrogen receptor (ER) and androgen receptor (AR) methods were replaced to address availability and cost issues.
 - An international validation study of the LUMI-CELL® Screening Assay, an estrogen receptor transcriptional activation assay. This validation study is the first joint effort by NICEATM/ICCVAM, ECVAM, and JaCVAM. Three laboratories (one in the United States, one in Japan, and one in Europe) are participating in the effort. The LUMI-CELL® standardization study, managed by NICEATM, was completed in July 2006.
- *ICCVAM International Collaborations:*

- The ICCVAM Genetic Toxicity Working Group will be commenting on proposed international validation studies on *in vivo* and *in vitro* Comet assays planned by JaCVAM and ECVAM.
- ICCVAM will be reviewing the validation status of the murine local lymph node assay (LLNA) for determining potency categories. In addition, the review will evaluate a “limit dose” proposal, evaluate a non-radioactive LLNA method, and develop LLNA performance standards.
- In November 2006, the “ICCVAM Biennial Progress Report” for 2004 – 2005 was released (<http://iccvam.niehs.nih.gov/about/annrpt/annrpt.htm>).

B. SACATM Discussion

Dr. Richard Becker asked about coordination with the OECD Endocrine Disruptor (ED) Task Force, specifically with the non-animal validation management group on activities related to the LUMI-CELL[®] ER screening assay. Dr. Stokes said this group has been kept informed of progress on the LUMI-CELL[®] assay and other ED related activities, such as the updated list of reference substances. Dr. George DeGeorge asked about the future of the MCF-7 cell proliferation assay nomination given the ongoing efforts to validate the LUMI-CELL[®] assay since they share the same general purpose of detecting estrogenic activity. Dr. Stokes replied that the MCF-7 cell proliferation assay could move forward depending upon comments made by SACATM and the public on the nomination and on the availability of funds. In addition, an outcome of the LUMI-CELL[®] validation will be the generation of performance standards against which other assays can be compared and validated in an expedited manner. Dr. DeGeorge agreed with the efficiency and utility of this approach and asked about the extent to which the “me too” or similar assay would have to be tested in multiple laboratories. Dr. Stokes said ICCVAM prefers that the assay be tested in multiple laboratories to demonstrate inter-laboratory reproducibility, which will be necessary if the “me-too” method is proposed for use in multiple laboratories.

V. ICCVAM NOMINATIONS

A. Presentation

Dr. Raymond Tice, NICEATM Deputy Director, reviewed the ICCVAM nomination and submission process and criteria for nomination priority setting. Next, he presented three nominations: (1) a nomination from CertiChem, Inc. (CCi) for the validation of a cell-based ER transcriptional activation (TA) test method measuring cell proliferation in MCF-7 cells, (2) an ICCVAM nomination for the study of *in vitro* cytotoxicity test methods for estimating starting doses for the acute oral systemic toxicity testing of mixtures, and (3) an ICCVAM nomination for the study of the most appropriate corneal holder and test substance solvent for the Bovine Cornea Opacity and Permeability (BCOP) test method. More information on nominations and submissions is available at <http://iccvam.niehs.nih.gov/nomsub/submission.htm>.

- *CCi Nomination.* In January 2006, ICCVAM-NICEATM received a submission from CCi entitled “Test Method Nomination: MCF-7 Proliferation Assay of Estrogenic Activity.” In brief, cells are incubated for one week with a test substance and the amount of DNA per well is quantified. Increases in DNA are used as a measure of cell proliferation which in this case is a surrogate marker of ER agonist activity. ER specificity is established if the known estrogen antagonist ICI 182,780 inhibits test substance-induced proliferation. One advantage of this test method compared to others is that it has been roboticized, making it amenable to high throughput testing. When CCi tested 40 ICCVAM recommended reference substances and compared the results to published ER activity they obtained greater than 90% concordance, sensitivity, and specificity. False negative and positive rates were less than 10%. ICCVAM considers the submission to be a high priority for validation and in October 2006 requested public comments on the CCi test method and proposed validation activity. Seven responses were received, six favorable and one expressing concern about the specificity of the

assay. Information on this nomination and the public comments can be found at <http://iccvam.niehs.nih.gov/nomsub/submission.htm>

- *ICCVAM Nomination - In vitro cytotoxicity test methods for estimating starting doses for the acute oral systemic toxicity testing of mixtures.* ICCVAM developed this nomination as a result of the recent peer review meeting (May 23, 2006) on the use of *in vitro* testing methods for estimating starting doses for acute oral systemic toxicity tests. The specific study objective is to determine the usefulness of the *in vitro* 3T3 NRU basal cytotoxicity test method for reducing and refining the use of animals for the acute oral toxicity testing of chemical mixtures. Test mixtures undergoing mandatory *in vivo* testing would be evaluated prospectively with the *in vitro* 3T3 NRU test method so no additional animal testing is required. The recommended priority is “high.”
- *ICCVAM Nomination - most appropriate corneal holder and test substance solvent for the BCOP test method.* The size and shape of the corneal holder used currently differs from the bovine cornea leading to potential damage of the cornea when it comes into contact with the holder. These issues were acknowledged during the evaluation of the BCOP method (“*In Vitro* Test Methods for Detecting Ocular Corrosives and Severe Irritants”), resulting in recommendations by the Ocular Expert Panel and ICCVAM to evaluate the impact of using a corneal holder that maintains normal corneal curvature on assay performance. Also, as part of the effort to refine the BCOP, ICCVAM proposes to evaluate the test method using alternative vehicles for the test substance (e.g., 0.9% NaCl instead of distilled water). ICCVAM considers this nomination to be “high” priority.

B. Public Comment

Dr. George Clark, Xenobiotic Detection Systems (developer of the LUMI-CELL[®] test method), commented that the false positive rate for the MCF-7 cell proliferation assay could be considered high (1 of 11). He is also concerned that funding the validation of multiple assays for the same purpose (e.g., detection of estrogenic activity) is a less efficient strategy of addressing public health issues compared to funding the validation of an assay that can be used in the near term and then moving on to address other important public health questions. While he understands the appeal of having multiple validated assays that measure the same general outcome, he questions whether this approach is fair to the individual or organization that developed the first validated method since that process takes the longest and is most expensive.

C. SACATM Discussion

CCi Nomination. Dr. Becker recused himself from discussions of the CCi nomination because his employer submitted formal public comments on the submission. Dr. Frank Barile asked for additional information on the indicators used in the assay and also on comparative performance when the assay is conducted manually and with robotics. Dr. Barile suggested testing more substances to better evaluate the false positive and false negative rates. Dr. Tice agreed that more substances need to be tested to address differences between the manual and robotics protocols and to evaluate assay performance. One of the reasons why this assay was given a draft “high” priority by ICCVAM is because of the potential to use robotics. Dr. Tice discussed the importance of using the ER antagonist to confirm that any observed cell proliferation is ER-mediated. This is important since non-ER based biological pathways can also induce cell proliferation. ER antagonists can be detected by assessing whether a particular substance inhibits the ability of estradiol to induce cell proliferation. Recognizing that many compounds may not act as complete agonists or antagonists, Dr. DeGeorge asked how the assay would respond to compounds with mixed agonist/antagonists activity. Dr. Tice agreed with the complexity of this issue, but noted that other assays, such as the LUMI-CELL[®] assay, face the same problem. Also, that when evaluating activity, especially antagonism, it is important to distinguish between true antagonism from compound-induced cell death. The LUMI-CELL[®] assay uses ATP and cell morphology to assess cell viability; the CCi assay would, due to the nature of the endpoint, use cell morphology.

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Dr. June Bradlaw commented that even the same cell line may differ between laboratories and asked what the source of MCF-7 cells would be (i.e., cloned cells obtained from CCI or another source). Dr. Tice responded that the laboratories participating in the validation effort obtained the cell line through CCI; however, the cells are included in the American Type Culture Collection (ATCC). Use of the appropriate quality controls is needed to assess cell line response both within a laboratory over time and across laboratories.

Dr. Grantley Charles asked whether more than one assay to address estrogenic activity is really needed. Dr. Stokes replied that one value for evaluating more than one assay is to compare performance of the assays. For example, is one better for identifying weakly estrogenic compounds or certain chemical classes? Dr. DeGeorge commented that all of the reference compounds need to be soluble in tissue culture media and asked about testing compounds that are not water soluble. Dr. Tice said all the reference compounds are soluble in either dimethyl sulfoxide (DMSO) or ethanol. Also, this assay is being proposed as a Tier 1 assay for EPA's efforts to screen for endocrine disrupting compounds. The Tier 1 battery will also have short-term *in vivo* studies and screening decisions will be made based on performance across the battery, not just a single assay. Each assay will have advantages and disadvantages. A benefit of having the same group of reference compounds tested in all the proposed assays is that it allows cross-platform comparisons.

Dr. Donald Fox asked if the assay measures cell number in addition to DNA content. Dr. Tice explained that earlier versions of the assay did measure cell number but that it is a labor-intensive procedure and measuring DNA levels is much more efficient. DNA content is widely accepted as a surrogate measure of cell number. A real issue for antagonists is to distinguish them from cytotoxic compounds. The LUMI-CELL[®] system addresses this issue by measuring ATP levels and by visual inspection of cell morphology and cell density. For the proliferation assay, visual observations of cell morphology and cell density are used. Dr. Roger McClellan asked what impact this nomination would have on LUMI-CELL[®] validation efforts. Dr. Stokes clarified that the LUMI-CELL[®] validation is scheduled to move forward in 2007 regardless of the priority assigned to the CCI nomination. Dr. Wilson asked for more information on how ICCVAM assigns a draft priority in the pre-screen evaluation. Dr. Tice said the draft priorities are largely based on the potential usefulness of the assay to address regulatory needs and the assay's ability to reduce, refine, or replace the use of animals. In addition, preliminary performance characteristics such as sensitivity, specificity, false negative, and false positive rates are considered. Dr. Fox asked whether any estrogenic endocrine disruptors are known to be inverse agonist. If so, then a genetically-modified cell line might be useful to identify these types of compounds. Although some members of SACATM had concerns about certain technical aspects of the assay, the group unanimously supported the "high" priority assignment of the nomination.

ICCVAM Nomination - In vitro cytotoxicity test methods for estimating starting doses for acute oral systemic toxicity testing of mixtures. Dr. Michael Dong was concerned that a lack of existing information on mixtures would limit the impact of pursuing this nomination. Dr. Tice responded that the nomination would make use of both historical data *and* data generated prospectively so that additional LD50 test would not have to be conducted for the purposes of validating the cytotoxicity test method for this use. Dr. Charles noted that many times a cytotoxicity assay is conducted during the course of a genotoxicity evaluation and he believes this would be an additional useful source of information that could negate the need to conduct new cytotoxicity 3T3 NRU tests. However, if that proves to be inadequate he concurs with the expert panel and considers the nomination to be high priority. Dr. Tice agreed that looking at this data would be a valuable exercise as would looking at cytotoxicity tests that have been conducted for other purposes. However, he believed there is merit to evaluating the 3T3 NRU cytotoxicity test method because it has a standardized protocol. There was consensus among SACATM that the nomination should be considered high priority.

ICCVAM Nomination - Most appropriate corneal holder and test substance solvent for the BCOP test method. Based on the recommendations of the ocular expert panel that reviewed this method, Drs. Charles and Bradlaw concur with the high priority. Dr. Barile sought confirmation that introduction of a new corneal holder does not mean the assay would have to go through the entire validation process again. Dr. Stokes clarified that it would not although studies would be conducted to confirm comparable or better performance than the current BCOP protocol. Dr. Barile also concurred with the high priority recommendation. Dr. Fox was very supportive of the high priority for this nomination and expected that it would reduce the false positive rate for the BCOP. Dr. Stephens agreed with the optimization efforts but does not want to see use of the BCOP held up until that process is complete. Dr. Tice explained that ICCVAM does not intend to delay transmitting test method recommendations pending the outcome of the optimization. There was consensus among SACATM that the nomination should be considered high priority.

VI. APPROACH FOR DEVELOPING THE NICEATM-ICCVAM 5-YEAR PLAN

A. Presentation

Dr. Schechtman presented the process for developing the NICEATM-ICCVAM 5-Year Plan and discussed how the plan would complement the “ICCVAM Mission, Vision and Strategic Priorities” document adopted by ICCVAM in 2004 (<http://iccvam.niehs.nih.gov/about/MisVisStrat.pdf>). Language in the fiscal year 2007 Senate Appropriations Report requests NICEATM-ICCVAM to create a 5-year plan to address the research, development, translation, and validation of new and revised non-animal and other alternative assays for integration of relevant and reliable methods into the federal agency testing programs. The process for developing the plan has numerous opportunities for input from the public and SACATM (Figure 1). As a starting point for discussion, ICCVAM highlighted areas identified in 2004 as the highest priority (presented in rank order) for alternative test method development:

1. Acute eye irritation/corrosion
2. Biologics/vaccines
3. Acute skin toxicity (including irritation/corrosion, sensitization, absorption)
4. Acute systemic toxicity (oral/dermal/inhalation)
5. Chronic toxicity/carcinogenicity
6. Reproductive/developmental toxicity
7. Endocrine disruptors
8. Neurotoxicity
9. Immunotoxicity

During the initial stage of SACATM and public input, ICCVAM requested comments on the following questions:

1. Considering available science and technology, what development, translation, and validation activities are most likely to have the greatest impacts within the next five years on refining, reducing, or replacing animal use?
2. What research and development activities hold the greatest promise in the long-term for

Figure 1. Abbreviated 5-Year Plan Timeline

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| <i>December 31, 2006</i> | Deadline for receipt of initial comments from the public and SACATM (71FR66172) |
| <i>May 2007</i> | Release of draft NICEATM-ICCVAM Plan for public and SACATM comment |
| <i>June 2007</i> | SACATM public meeting to discuss draft NICEATM-ICCVAM Plan; deadline for receipt of comments from the public and SACATM on the draft |
| <i>August 2007</i> | ICCVAM approves final NICEATM-ICCVAM Plan and sends to agencies for concurrence |
| <i>November 2007</i> | Public release of final NICEATM-ICCVAM Plan |

refining, reducing, or replacing animal use?

3. What are appropriate measures for evaluating progress in enhancing the development and use of alternative test methods?

B. Public Comment

Ms. Sara Amundson, Humane Society Legislative Fund, talked about her organization's role in garnering budget support for ICCVAM-NICEATM activities. She is a strong supporter of developing a 5-year plan. In particular, she emphasized the importance of engaging individual agencies. She noted that research efforts within agencies may or may not be coordinated with translation efforts. Ms. Amundson suggested that instead of requesting public comments on a draft list of priorities, that ICCVAM-NICEATM organize a workshop or meeting of stakeholders to encourage in depth discussions. Also, she encouraged ICCVAM-NICEATM to engage ECVAM and JaCVAM in developing the plan.

C. SACATM Discussion

Dr. Daniel Marsman supported developing a 5-year plan, but said he would need additional information before being able to provide meaningful feedback on the priority list that covers the majority of current toxicological endpoints. Specifically, he would like to see (1) a description of the specific endpoints and assays being addressed, (2) the regulatory goal or purpose of each assay, and (3) the critical regulatory information provided by the current *in vivo* model. For example, *in vivo* and *in vitro* assays for acute skin toxicity already exist and progress is being made on validating the alternatives. He would also like to see more attention paid to alternative methods that could be useful for estimating risk and exposure rather than only predicting hazard. Dr. Fox had questions about the ICCVAM priority list. He asked for clarification on what aspect of human health protection is sought (i.e., the general population? susceptible subpopulations?). He believes potential human health impact should be the foremost consideration. Also, he wondered whether the focus is on acute or chronic toxicity, with acute toxicity being easier to address in alternative models. Other toxicities may simply be too complicated to adequately address *in vitro*. For example, neurotoxicity endpoints, such as sensory input, cognitive function, and pain and suffering cannot be assessed *in vitro*. In addition, the relevance to humans of alternative species is unknown. Other important factors to keep in mind when prioritizing are predictability and cost of the assay. He questioned the ranking of the list and agreed that a presentation of assays, endpoints, and regulatory targets for each of the priority areas would be helpful. Dr. McClellan thought the priority areas should not just be a function of how they address the 3 R's, but also why they are important for promoting public health. He suggested including in the plan a couple of case studies that incorporate the cost of developing a particular method. Several others SACATM members agreed that human health protection is the priority and that animal welfare concerns should be layered over that. Dr. Barile thought assay prioritization should be left to ICCVAM-NICEATM as they are in the best position to understand how factors such as budgetary constraints impact alternative test method development. For example, is an area at the research and development stage or are there promising methods that are ready for standardization and validation? These results could then be put into a matrix and specific priorities would be apparent. Dr. DeGeorge believed prioritization should reflect discussions based on weighing animal use and distress versus human impact and effort (e.g., cost-benefit and resources). Also, toxicology has evolved over the past decades and the future is more in the realm of mechanisms and pharmacology. With this in mind, he thought perhaps that the focus should be on refinement. From this, he believes reduction and replacement will follow. To aid transparency, Dr. Becker suggested the agencies describe the testing and testing batteries for each of the priority areas and how these lead to the protection of human health and the environment. Also, for each, a description of what is needed to address the 3 R's for a test or tests battery. Dr. Becker agreed that a workshop with stakeholders would be very valuable. Several other SACATM members had questions about how the priority list and relative ranking were developed. Dr. Freeman summarized that SACATM suggests the plan contain more transparency on the how the priority list was developed.

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Dr. Stokes added that all of the areas identified on the priority list have regulatory testing requirements because their relevance for protecting public health has already been established. The challenge is to improve the predictability of toxicology tests while promoting the 3Rs. The top 3 – 4 areas on the priority list are there because those tests are associated with significant unrelieved pain and distress in animals. With respect to the priority list, Dr. Marilyn Wind, CPSC, explained that ICCVAM reviewed the input from agencies on alternative test methods priorities and evaluated this list in light of the numbers of animals used, the amount of pain and distress, etc. For this reason, ocular toxicity is at the top of the list. ICCVAM intends to look at short, medium, and long-term goals and is asking SACATM to help identify which types of activities would lead to immediate versus long-term impacts. Dr. Charles thought that many of the priority areas are long term because they address chronic toxicity and complex systems. He agreed that more detail specifically is needed regarding what is being addressed for the priority areas. Dr. DeGeorge thought empowering ICCVAM to mandate that member agencies accept the test methods considered by ICCVAM could have a significant impact on the use of alternatives in the short run. In some cases, ICCVAM determines that a test method is validated, but an ICCVAM member agency does not necessarily accept that test method or accept it readily.

Dr. Stephens encouraged ICCVAM to obtain broad stakeholder input and consider interactions with ECVAM and JaCVAM when developing the plan. He also suggested that consideration be given to investing resources into issues that affect *in vitro* methods in general (i.e., metabolism, lack of reference human data) rather than into developing assays endpoint by endpoint. He agreed that budgetary constraints impact prioritization such that not only could the magnitude of a particular effort be impacted, but also the types of efforts considered as priorities. He did not believe that there is a trade-off between promoting public health and developing 3R approaches. Human health protection is the primary consideration as alternatives have to be at least as good as the existing method. Dr. McClellan believed validating test methods against human data is a greater priority than validating an alternative test method against an existing regulatory test.

Dr. Cunningham agreed that agencies might need to reevaluate whether the appropriate endpoints are being addressed in regulatory testing because many have been required for decades. For example, is it still appropriate to classify toxicity by tissue or organ system? Could better use be made of new information, including genomes? One hurdle to developing assays based on new knowledge and techniques is the lack of clarity as to whether they would be used for screening purposes or as regulatory tests. She thought that methods that incorporate toxicogenomics and systems biology could be ready for use within the next 5 years. Dr. Barile added that encouraging research grants, such as small grants development, would be a key component to ICCVAM achieving its goals and promoting the development of new ideas.

Dr. McClellan asked about using animal usage numbers to evaluate the impact of alternative test methods. Dr. Wind responded that evaluating animal usage is very difficult because this information is typically not reported to agencies. Also, rats and mice are not included in the USDA annual reports on animal usage. Dr. Stephens recognized the challenges of identifying accurate and reliable measures of impact but believed it is crucial. He did not believe national statistics are as informative as assessing the impact of adopting a specific test method. For example, finding out the extent to which an alternative test method is used by industry after ICCVAM determines a test method is scientifically validated. He suggested including measures of regulatory acceptance in evaluation. If good quality animal usage numbers are not obtainable, Dr. Flournoy suggested that documenting the number of validated assays and the regulatory acceptance of them could be markers for evaluation of impact. Dr. Schechtman thought that one of the main hurdles for translating research methods into something that can be evaluated for validation status is that the test method development effort often stops at the publication stage. This hurdle also makes it difficult to evaluate implementation.

VII. ECVAM UPDATE

A. Presentation

Dr. Thomas Hartung, Head of ECVAM, provided a brief update of ECVAM. Major points were:

- ECVAM has validated ~25 methods since its establishment in 1991.
- Many of the activities during 2003 – 2007 occurred in response to the 7th Amendment of the Cosmetic Directive and REACH legislation (Registration, Evaluation, Authorisation and Restriction of Chemicals). ECVAM is responsible for coordinating the test strategy development for REACH involving more than 200 experts from chemical industry and regulators and expects to have a draft strategy ready by April 2007. Future activities will expand to methods used to assess the safety of drug, vaccine, food and nanoscale materials.
- REACH allows for the use of “suitable” *in vitro* methods. In this case, suitable means sufficiently well-developed according to internationally agreed test development criteria, such as the ECVAM criteria for the entry of a test into the pre-validation process. A suitable (or “adequate”) *in vitro* method does not have to be validated to the extent that its predictive capacity, range of applicability, and minimum performance standards are established. ECVAM expects that approximately 100 *in vitro* methods will be deemed “suitable” for REACH in 2009.
- Tests for the following have been validated recently or are at advanced stages of development and evaluation: topical toxicity (skin and eye irritation), systemic toxicity (use of cytotoxicity to predict starting doses for acute toxicity, cytotoxicity to predict non-toxic chemicals, tiered test for dermal and inhalation toxicity), mutagenicity and cancer (cell transformation assays, micronucleus, Comet assay for detecting DNA damage in single cells, skin models for genotoxicity), sensitization (LLNA cut down approach and non-radioactive variants, monocyte/dendritic cell assays, peptide-binding assay), and ecotoxicology (acute ecotoxicity, fish egg/embryo test, bioaccumulation). ECVAM is also pursuing alternative methods for reproductive toxicity (extended one-generation assay, various endocrine disrupter tests). This area is of high impact, as an estimated 80% of animals in REACH will be used to assess reproductive toxicity. As part of its integrated project ReProTect, ECVAM is in the process of developing and validating a test battery covering the reproductive cycle.
- So far, the testing strategy being developed under REACH does not include requirements for neurodevelopmental toxicity, endocrine disruption, respiratory irritation and sensitization, and a second species in the two-generation reproduction study. The impact of utilizing replacement and reduction methods as part of REACH could result in 8 million fewer animals used.
- 160 methods are under validation and ECVAM expects a total of 40 methods to be validated by the end of 2009. An impact analysis suggests the use of these alternatives could decrease animal usage by 50% with an estimated 20% additional reduction occurring when quantitative structure-activity relationship (QSAR) analysis is used. Dr. Hartung explained that the recent pace of success at ECVAM is a result of people understanding that the process can work and engaging in the effort as well as the synergy between legislative pressure and technological advances. There is also the financial support to ensure that validation is not a bottleneck for the availability of alternative methods.

B. SACATM Discussion

Dr. Karen Hamernick, EPA, asked how industry would evaluate false positive results. Dr. Hartung replied that the testing strategies aim to reduce this problem by not basing interpretation solely on an animal experiment, but by using a tiered strategy that screens for substances of concern. These substances would go on for *in vivo* testing. This approach is expected to reduce the number of false positives. Dr. Becker asked if there is concern about using a “suitable” method rather than one that has been completely validated. It appears that the validation bottleneck was solved by simple redefining what

is required before a test method can be accepted. Dr. Hartung said he is not particularly satisfied with the change in approach either, but noted that most animal tests have not undergone scientific validation either. In addition, the "suitable" *in vitro* methods are being used in a weight of evidence evaluation only. Dr. Becker asked for confirmation that a positive *in vitro* result can lead to classification for a substance, but negative results would require further testing. Dr. Hartung responded that increasingly negative results are accepted as well. This acceptance is necessary because the frequency of detecting a "toxic" compound for a given endpoint is low (e.g., 20% for ocular toxicity, 7% for skin irritation). If negative results need to be followed up with animal tests, then there would not be a significant decrease in animal usage (e.g., 93% of compounds would still need to be tested for skin irritation).

VIII. JACVAM UPDATE

A. Presentation

Dr. Hajime Kojima, Director of JaCVAM, presented an overview of the organization and mission of JaCVAM.

- JaCVAM was founded by Dr. Yasuo Ohno in November 2005 and is part of the National Institute of Health Sciences (NIHS), National Center for Biological Safety and Research (NCBSR) in the Division of Pharmacology.
- The JaCVAM Steering Committee makes decisions on the need for validation or peer review for a particular new or revised method. JaCVAM utilizes an advisory board for organizational advice. An oversight committee and peer review panels are the primary entities involved in the scientific validation and evaluation of a method. International study management teams plan and oversee the conduct of validation studies.
- The following test methods are currently undergoing validation or peer review:

| Test Method | Material | Current activities |
|---------------------|---|----------------------------|
| Phototoxicity | Yeast-RBC | Peer Review in progress |
| Skin sensitization | LLNA-DA | Validation in progress |
| | LLNA-BrdU | Validation in progress |
| | h-CLAT | Pre-validation in progress |
| Corrosivity | Culture model | Peer Review in progress |
| Skin irritation | Culture model | Planning on Peer Review |
| Endocrine disrupter | LUMI-Cell [®] , CERI-estrogen reporter assay | Planning on Validation |
| Mutagenicity | Comet assay (<i>in vivo</i> or <i>in vitro</i>) | Planning on Validation |

B. SACATM Discussion

Dr. Hamernick asked how many regulatory agencies exist in Japan. Dr. Kojima said there is only one. Dr. Schechtman welcomed JaCVAM into the "validation fold" with ICCVAM and ECVAM and looked forward to collaboration.

IX. ADJOURNMENT

SACATM and ICCVAM recognized the retirement of Dr. Schechtman. Dr. Stokes presented a Certificate of Appreciation to Dr. Schechtman as a token of appreciation from ICCVAM and NICEATM for the 5 years that he has served as the Chair of ICCVAM, and the nine years that he has served as the FDA's Principal Agency Representative on the ICCVAM. Dr. Schechtman expressed his gratitude to

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ICCVAM and NICEATM for the opportunity to participate and contribute to the important mission of these organizations. The following agenda topics were not discussed due to time constraints:

- Status of NTP Roadmap Initiative on High Throughput Screening (HTS)
- Follow-up on ICCVAM Report Recommendations

The meeting adjourned at 4:15 PM.