Scientific Advisory Committee on Alternative Toxicological Methods
Minutes from the June 12, 2007 SACATM Meeting

MEETING OF THE
SCIENTIFIC ADVISORY COMMITTEE ON ALTERNATIVE TOXICOLOGICAL METHODS
JUNE 12, 2007

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").

3Rs	Replacement, reduction, refinement (causing less pain and distress) in the use of animals for toxicologic testing
ATSDR	Agency for Toxic Substances and Disease Registry
CPSC	Consumer Product Safety Commission
DOT	Department of Transportation
EPA	Environmental Protection Agency
ESAC	ECVAM Science Advisory Committee
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
REACH	Registration, Evaluation, Authorisation and Restriction of Chemical substances
SACATM	Scientific Advisory Committee on Alternative Toxicological Methods
USDA	U.S. Department of Agriculture

II. ATTENDANCE

SACATM met on June 12 2007, at the Marriott Bethesda North Hotel and Conference Center, Bethesda, MD, 20852 The following individuals attended the meeting:

SACATM

James Freeman, Ph.D., ExxonMobil Biomedical Sciences, Inc., *Chair*
Frank Barile, Ph.D., St. John's University
Richard Becker, Ph.D., American Chemistry Council

ICCVAM Primary Representatives

George Cushmac, Ph.D., Department of Transportation
Susan Fitzpatrick, Ph.D., FDA
Jeanne Goshorn, Ph.D., National Library of Medicine

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
Helen Diggs, D.V.M., University of California - Berkeley
Michael Dong, Ph.D., California Department of Pesticide Regulation
Marion Ehrich, Ph.D., VA-MD Regional College of Veterinary Medicine
Daniel Marsman, D.V.M., Ph.D., Procter & Gamble Company
Roger McClellan, D.V.M. (retired)
Annie Qu, Ph.D., Oregon State University

SACATM Working Group
A. Wallace Hayes, Ph.D., Harvard School of Public Health
Martin Stephens, Ph.D., Humane Society of the United States

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

NIEHS/NIH Staff
John Bucher, Ph.D., D.A.B.T.
Raj Chhabra, Ph.D., D.A.B.T.
Allen Dearry, Ph.D.
Sally Fields
Debbie McCarley
Sheila Newton, Ph.D.
Barbara Shane, Ph.D. D.A.B.T.
Raymond Tice, Ph.D.
Mary Wolfe, Ph.D. (*executive secretary*)

Public
Aysha Akhtar, M.D., Physicians Committee for Responsible Medicine
Nancy Beck, Ph.D., Physicians Committee for Responsible Medicine
Karen Hamernik, Ph.D., EPA
Moiz Mumtaz, Ph.D., ATSDR
Richard McFarland, Ph.D., FDA
Paul Nicolaysen, V.M.D., NIOSH
Alan Poland, M.D., NCI
William Stokes, R.A.D.M., D.V.M., NIEHS
Margaret Snyder, Ph.D., NIH
Marilyn Wind, Ph.D. CPSC, *ICCVAM Chair*

Other Federal Staff
Kristine Hatelid Ph.D. (CPSC)
Paul Howard, Ph.D. (FDA)
Abby Jacobs, Ph.D. (FDA)
Joanna Matheson, (CPSC)

ILS staff (NICEATM support contractor)
David Allen, Ph.D.
Jeffrey Charles, Ph.D., M.B.A., D.A.B.T.
Douglas Winters, M.S.

Linda Loretz, Cosmetic Toiletry and Fragrance Association
Nina Mak, American Anti-Vivisection Society
Robert Scala, Ph.D. (Exxon, retired)
Jerry Smrchek, Ph.D., EPA

Jay Birnbaum, Jay Birnbaum Consulting
Karen Brown, Ph.D., DRL Pharma and Pair O' Docs Enterprises
Rodger Cullen, Ph.D., Institute for In Vitro Sciences
Erin Hill, Institute for In Vitro Sciences
Melissa Kirk, MB Research Laboratories
Sue Leary, Alternatives Research & Development Foundation
Kristie Stoick, Physicians Committee for Responsible Medicine
Loree Talby, Humane Society of the United States
Catherine Willett, Ph.D., People for the Ethical Treatment of Animals

III. WELCOME, INTRODUCTIONS, AND RECOGNITION OF RETIRING MEMBERS

Dr. Freeman, SACATM Chair, called the meeting to order at 8:30 a.m. Individuals in the room introduced themselves. Dr. Dearry, Interim Associate Director of the NTP, made opening remarks. He said he appreciated the commitment of the members to SACATM and noted an important topic for discussion would be the draft NICEATM-ICCVAM 5-Year Plan.

Dr. Wind, ICCVAM chair, welcomed everyone and said she appreciated SACATM's input to ICCVAM and acknowledged the public's participation and input on the 5-year plan at the Town Meeting yesterday.

Dr. Wolfe, executive secretary, read the conflict of interest statement for SACATM. She noted that Drs. Marsman and DeGeorge would recuse themselves from committee's discussion of the LLNA test.

IV. ICCVAM-NICEATM UPDATE

A. Presentation

Dr. Stokes, NICEATM Director and ICCVAM Executive Director, provided an overview of ICCVAM/NICEATM activities since the November 30, 2006 SACATM meeting and highlighted several topics on the current meeting's agenda.

1. Acute Systemic Toxicity
   The ICCVAM test method evaluation report, *The Interagency Coordinating Committee on the Validation of Alternative Methods Test Method Evaluation Report: In Vitro Cytotoxicity Test Methods for Estimating Starting Doses for Acute Oral Systemic Toxicity Tests,* is published. ICCVAM issued test method recommendations on two *in vitro* test methods proposed for estimating starting doses for acute oral systemic toxicity tests. These *in vitro* basal cytotoxicity test methods, where appropriate, should be considered and used before using animals for acute oral toxicity testing. The report also provides recommendations for: (1) standardized protocols that should be used when performing the test methods, (2) future studies to improve the usefulness of *in vitro* assays for assessing acute oral systemic toxicity, and (3) performance standards that can be used to assess the performance of similar test methods.

ICCVAM and NICEATM's planned activities on acute systemic toxicity are to (1) stimulate industry's submission of comparative *in vitro* and *in vivo* data, (2) evaluate mixtures in one or more of these two *in vitro* test methods, (3) convene an international workshop on Advancing In Vitro Approaches and Humane Endpoints for Systemic Toxicity, (4) identify existing *in vivo* reference data, and (5) evaluate *in silico* approaches.

2. Ocular Toxicity Testing
   The test method evaluation report, *Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Test Method Recommendations on In Vitro Test Methods for Identifying Ocular Corrosives and Severe Irritants* is published. ICCVAM recommended that four alternative test methods (Bovine Corneal Opacity and Permeability (BCOP) Test, Isolated Chicken Eye (ICE) Test, Isolated Rabbit Eye (IRE) Test, and the Hen's Egg Test - Chorioallantoic Membrane (HET-CAM) Test Method) should be considered for possible use prior to using rabbits for ocular testing. Two of the methods, BCOP and ICE, are considered to have sufficient data and performance to substantiate their use for testing some types of substances for regulatory hazard classification purposes. The report also provides recommendations for: (1) standardized protocols that should be used when performing the four different test methods, (2) studies to further optimize and improve the performance of each test method, and (3) a list of reference substances for use in future validation studies. Ocular testing is one of the top six most commonly performed product safety tests.

   Future activities include to (1) evaluate a holder that maintains normal corneal curvature in the BCOP, (2) develop guidance for using histopathology as part of the decision criteria for identifying ocular corrosives or severe irritants (BCOP, IRE, ICE, rabbit), (3) encourage industry use and data submission to expand current validation databases and (4) review the routine use of topical anesthetics and systemic analgesics in the traditional rabbit eye test.

3. Alternative Methods for Botulinum Toxin Testing
   ICCVAM, NICEATM, and ECVAM held a scientific workshop on Alternative Methods for Botulinum Toxin Testing on November 13-14, 2006, in Silver Spring, MD. The purpose of the workshop was to review the state-of-the-science regarding alternative methods for reducing, replacing, and refining (causing less pain and distress), otherwise known as the 3Rs, the use of mice for botulinum toxin toxicity testing and to identify and prioritize future research, development, and validation efforts needed to further the use of alternatives for this purpose. The presentations and report from the workshop are on ICCVAM's website at *http://iccvam.niehs.nih.gov/methods/biologics/biologics.htm*

4. Endocrine Disruptor Test Methods
   Dr. Stokes outlined an international validation study jointly sponsored by NICEATM, JaCVAM, and ECVAM to evaluate the ability of the LUMI-CELL® Estrogen Receptor (ER) transcriptional activation assay, which was developed by Xenobiotic Detection Systems (XDS), Inc., to detect substances that have the ability to act as an ER agonist and/or antagonist. Standardized protocols for both endpoints have been established. The U.S. and Japanese laboratories began their studies in April 2007 and ECVAM will conduct an "in house" validation study.

The OECD is developing test guidelines for the rat uterotrophic and the Hershberger assays. ICCVAM provided extensive comments on the validation status and protocol issues for both assays to the U.S. National Coordinator to the OECD, which were forwarded to the OECD.

5. Pyrogenicity Test Methods:
   ICCVAM convened an independent peer panel on February 6, 2007, at the NIH in Bethesda, MD. Thirteen scientists from five countries participated on the panel. Dr. Stokes noted that Dr. Richard McFarland, Chair of the ICCVAM Pyrogenicity Working Group, and Dr. Karen Brown, Peer Review Panel Chair, would discuss the outcome of the review later.

6. LLNA: An Alternative Test Method for Allergic Contact Dermatitis Testing
   Dr. Stokes provided a historic background to the Local Lymph Node Assay (LLNA). He said ICCVAM evaluated the test in 1998, it was accepted by U.S. regulatory agencies in 1999, and an implementation workshop was held in 2000. In 2002, the OECD released a test guideline for the LLNA, which indicated that the LLNA is a valid substitute for the guinea pig tests in most situations for assessing allergic contact dermatitis. This test is one of the six most commonly performed product safety tests, and compared to guinea pig tests, its use eliminates pain and distress while using fewer animals.

   In January 2007, the CPSC requested that NICEATM and ICCVAM assess the validation status of (1) the LLNA as a stand-alone assay for potency determination for classification purposes; (2) modified LLNA protocols; (3) the LLNA limit test; (4) the use of LLNA to test mixtures, aqueous solutions, and metals; and (5) the applicability domain for LLNA. In May 2007, NICEATM published a *Federal Register* notice requesting comments on the nomination, data for the nominated activities, and nominations for possible experts to serve on a review panel. Dr. Stokes noted that SACATM would be asked to comment on the LLNA nomination at the meeting today.

7. Genetic Toxicity Test Methods
   Dr. Stokes provided updates on three genetic toxicity tests: the *in vitro* micronucleus assay, cell transformation assays, and the Comet assay.

   a. Draft OECD TG 487: *In Vitro* Micronucleus Assay
      ICCVAM submitted comments to OECD via the U.S. National Coordinator. OECD plans to establish an *ad hoc In Vitro* Micronucleus Expert Group to revise the draft.

   b. Draft OECD Detailed Review Paper 31: Cell Transformation Assays
      ICCVAM submitted comments to OECD via the U.S. National Coordinator asking for a deferral of the review paper to allow incorporation of current validation results.

   c. JaCVAM-sponsored International *In Vivo* and *In Vitro* Comet Assay Validation Studies
      The ICCVAM Genetic Toxicity Working Group provided comments on the draft protocols and study design.

8. Dermal Toxicity

In 2006, ECVAM completed an *in vitro* skin irritation validation study of EpiDerm™ and EPISKIN™, based on the European Union hazard classification scheme for dermal irritants. In 2007, ECVAM's Scientific Advisory Board recommended that EPISKIN™ be considered a full replacement for the rabbit skin irritation test. In the future, ICCVAM plans to evaluate the usefulness of these methods for the U.S. hazard classification schemes used by EPA, the Federal Hazardous Substances Act, and the United Nations Globally Harmonized System of Classification and Labeling of Chemicals.

Also, ICCVAM plans to determine what happens when *in vivo* rabbit dermal corrosives that are not correctly identified by *in vitro* dermal corrosivity test methods (i.e., are classified as false negatives) are tested in these *in vitro* dermal irritation test methods. Presently, these false negative substances would be correctly identified as corrosive in an *in vivo* rabbit skin irritation test, and the in vitro dermal irritation test may not detect these as corrosives.

9. NICEATM-ICCVAM Five-Year Plan
   The draft plan was made available publicly for comment on May 7, 2007, on the NICEATM-ICCVAM website. A request for public and stakeholder comments were also welcomed at a June 11 public town meeting, and time is set aside at this meeting to discuss the draft plan.

10. ICCVAM-NICEATM Outreach Activities
    ICCVAM is focusing on promoting awareness of alternative methods and providing education on test method uses.
    - ICCVAM/NICEATM presented seven posters at the March 2007 Society of Toxicology meeting in Charlotte NC on the following topics: acute oral and systemic toxicity, *in vitro* and *in vivo* assays to measure ocular irritation hazards. NICEATM presented a special session, *Using Animals for Toxicological Research and Testing: Best Practices for Assuring Compliance with Animal Welfare Regulations, Policies and Guidelines*. Also, ICCVAM-NICEATM distributed brochures and flyers to increase awareness about the June 11 town meeting on the draft 5-year plan.
    - Representatives from NICEATM-ICCVAM will attend the 6th World Congress on Alternatives and Animal Use in the Life Sciences in August 2007 in Tokyo, Japan and will make eight platform presentations and present 13 posters.
    - The NICEATM-ICCVAM website now lists all NICEATM-ICCVAM publications, applicable federal and international regulatory documents, relevant *Federal Register* notices, and public comments relating to ongoing testing.

11. NICEATM-ICCVAM Progress
    Dr. Stokes highlighted some ICCVAM accomplishments. ICCVAM has evaluated over 150 test methods since 1997 and the committee's recommendations have had an impact on the alternative test methods recommended and/or adopted internationally for four of the six most common product safety tests. ICCVAM's recommendations have addressed advancement of alternative methods in the areas of research, development, translation, and validation and performance standards to expedite validation studies and regulatory acceptance. ICCVAM has also provided guidance on the validation and regulatory acceptance of new, revised, and alternative methods and plans to hold a 10-year Anniversary Symposium in 2008.

V. NICEATM-ICCVAM 5-Year Plan

A. Presentation

Dr. Wind briefly reviewed the five-year plan, which was prepared in response to request from the U.S. House and Senate appropriations Committees. Congress asked ICCVAM to create a five-year plan, building on the NTP Roadmap, that encompasses research, development, translation, and validation of new and revised *in vitro* and other alternative assays for integration into federal agency testing program. The plan should identify areas of high priority for these assays or batteries of assays to create a path forward for the 3Rs when it is scientifically valid and appropriate. The report must be submitted to the NIH Budget Office by September 15, 2007, in order to complete clearance by a congressional deadline of November 15, 2007. The report should be limited to about 20 pages, must be understandable to policy makers, and budget-neutral.

A *Federal Register* notice was published in May 2007 requesting public comment on the draft NICEATM-ICCVAM Five-Year Plan and announcing the June 11 town meeting. The meeting will be a public forum for comment on the plan.

The plan identifies priority areas for future research and describes outreach and communication approaches to foster acceptance and appropriate use of alternative test methods. It has four chapters: (1) Research, Development, Translation, and Validation Activities for Priority Test Methods to Reduce, Refine, and Replace Animals in Regulatory Testing, (2) Incorporating New Science and Technology, (3) Fostering Acceptance and Appropriate Use of Alternative Test Methods, and (4) Developing Partnerships and Strengthening Interactions with ICCVAM Stakeholders. Comments from SACATM and the public will be considered in finalizing the plan.

B. Town Meeting

Dr. Robert Scala provided a brief presentation on the Town Meeting for the Five-Year Plan for which he served as moderator. More than 60 members of the public attended along with 25 members of ICCVAM and their respective program offices, and 9 members of SACATM. He noted that Dr. Stokes described the process for developing the plan and Dr. Wind presented the draft plan.

Four organizations provided oral comments: People for the Ethical Treatment of Animals, Physicians Committee for Responsible Medicine, Alternatives Research & Development Foundation, and Institute for *In Vitro* Sciences. Dr. Scala identified points from the comments relative to development of the plan:

- There is support for NICEATM and ICCVAM, but there is concern about the scope of their activities. ICCVAM should take a leadership role in the development and validation of alternative test methods within the United States.
- The five-year plan needs to identify deliverables with a timeline and serve as a blueprint for future activities. A web-based scorecard was suggested to increase transparency of ICCVAM's activities.

- The plan lacks a strategy for setting priorities. It should also have a means for evaluating success.
- The plan should describe how it would incorporate new science or technology.
- ICCVAM should focus on replacement rather than reduction and refinement of animal use.
- ICCVAM should evaluate its peer review process to ensure that it meets the intended goals and that the participants understand the process and their charge.
- ICCVAM should develop a means to expedite review of ESAC-endorsed test methods to prevent duplicative efforts.
- NICEATM/ICCVAM should be more proactive in developing ways to help prioritize funding within agencies. ICCVAM should encourage funding of research on alternatives.

Dr. Scala believes that ICCVAM has the experience, skill and dedication to be a leader in furthering the field of alternatives, but noted that the committee is somewhat constrained by its congressional mandate. ICCVAM must be empowered to move these efforts forward so progress can be made.

C. SACATM Working Group on the Five-Year Plan

Dr. Becker, chair of the working group, outlined the charge to the SACATM Five-Year Plan Working Group (FYPWG) and recognized its members namely: Drs. Mary Jane Cunningham, Helen Diggs and himself from SACATM and Drs. A. Wallace Hayes and Martin Stephens, past SACATM members. Their charge was to review how well the plan addressed the following objectives: (1) research, development, translation, and validation of new and revised non-animal and other alternative assays for integration into federal agency testing programs and (2) identification of areas of high priority for new and revised non-animal and alternative assays for replacement, reduction, and refinement (less pain and distress) of animal tests.

The FYPWG evaluated the draft based on whether it was comprehensive, strategic, and sufficiently detailed and whether the plan (1) had clearly defined priorities and milestones, (2) described clearly defined roles of the involved groups, (3) identified barriers and gaps, and (4) addressed communication with stakeholders.

The FYPWG thought the plan was comprehensive and was impressed with the compilation of ongoing research and development activities, methods standardization, methods development activities, and newer technologies such as high throughput screening. They recognized the critical role of NICEATM and ICCVAM in actualizing alternative test methods within federal programs.

The FYPWG thought the draft plan fell short strategically. They recommended that ICCVAM identify 2-3 high priority areas and provide a detailed plan to accomplish these activities in those areas. They encouraged NICEATM/ICCVAM to define and address both technical and procedural challenges and to describe their position strategically in five years. They suggested that the plan include timelines and milestones for the 2-3 highest priorities and identify the lead agency responsible for directing activities along the “pipeline” of activities toward research, development, translation, validation and regulatory acceptance.

The FYPWG determined that the draft plan did not clearly identify the gaps and barriers for methods development, validation, and adoption and appeared be weighted toward research and development. They suggested including a table that identified the methods previously reviewed by ICCVAM and the subsequent agency action for each. This information could be used to identify any gaps or barriers for the development of current or future methods.
The FYPWG recognized and commended NICEATM and ICCVAM for their extensive outreach efforts to obtain comment and input for the plan. They recommended that the plan include elements to engage stakeholders on an ongoing basis.

In conclusion, the FYPWG felt it incumbent upon the ICCVAM agencies to fully embrace the 3Rs and to exert the leadership needed to assure that the validated methods approved by the efforts of NICEATM and ICCVAM are actualized into regulatory testing frameworks as soon as practical.

Dr. Stevens added that the plan should indicate that ICCVAM would take a leadership role on certain priorities, but that the United States should take advantage of the work on alternatives being done overseas.

D. Public Comments

1. Dr. Catherine Willett representing People for the Ethical Treatment of Animals (PETA) said the report from the SACATM Working Group was excellent and PETA agreed with the report. Her major concerns are that new test methods frequently involve the lives of tens of thousands of animals, there is a lack of leadership by ICCVAM, and federal agencies are reticent to adopt *in vitro* assays that have been validated in Europe. ICCVAM's role in the U.S. is crucial and essential; however, over the past 10 years, Dr. Willett felt that ICCVAM has not fulfilled its mandate and has had poor leadership. She noted that ICCVAM consists of 15 agencies each with different needs and interests. The lack of leadership is reflected in the five-year plan, which does not make a commitment to the 3Rs. She suggested that Congress requested the 5-year plan because the public is disappointed with ICCVAM's progress. She said the U.S. needs an expedited process for acceptance of methods approved internationally. She noted that for ICCVAM to accept an *in vitro* method it has to be as good or better in predicting human health effects and ecological risk than *in vivo* animal studies; however, animal methods are not held to the same validation standards as *in vitro* methods and they are believed valid because they have been used historically. It is important that ICCVAM carry out its activities more efficiently so that *in vitro* methods can be accepted and used.

   In response to a question from Dr. McClellan about ICCVAM's use of resources, Dr. Willett thought ICCVAM's resources could be better spent by changing its priorities. Specifically, she thought ICCVAM's lengthy review of test methods that have already been reviewed in Europe could be expedited. She suggested better communication and coordination of reviews between Europe and ICCVAM to prevent redundancy. In addition, she suggested that additional resources for the development of *in vitro* assays are needed in the United States.

2. Dr. Karen Brown, DRL Pharma and Pair O' Docs Enterprises, said she had worked in the animal health industry for more than 35 years where she developed *in vitro* assays. She worked closely with government agencies to try to reduce, refine, and replace animal tests. She chaired the pyrogenicity review panel and believes the ICCVAM process is workable; however, some modifications are needed. First, the charge to the pyrogenicity panel, which was to examine the validation status of five *in vitro* assays proposed as replacements for the rabbit pyrogenicity test, was overwhelming for the one day allotted for the review. She thought that the ICCVAM process might be modified to require companies, which are interested in utilizing a specific test, to evaluate and validate it. She suggested that the review panel for a method could then be comprised of independent scientists with applicable experience as well as stakeholders from industry and the regulatory agencies.

   She pointed out that most, if not all, current animal tests for product testing have not gone through as rigorous a validation process as was used for the pyrogenicity assays, although such animal tests are considered the "gold standard." She noted that many animal tests could never attain the level of reproducibility that is achievable with *in vitro* assays because of individual animal variability. If the objective is to validate all *in vitro* assays against current animal tests, then the goal to replace, reduce, and refine animal tests may never be reached. She said accurately validated *in vitro* assays can be very reproducible and accurate, but the methodology used for their validation is key. There must be a very "tight" protocol that defines every reagent, time interval, and test condition in order to get reliable results.

3. Dr. Aysha Akhtar representing the Physicians Committee for Responsible Medicine said there is an incredible disconnection between the validation processes for the non-animal versus animal methods because there has been little or no validation of the animal tests that are considered the "gold standard." The systematic reviews and meta-analyses published in the clinical literature conclude that animal data do not predict human outcomes in a variety of medical fields. She said clinicians do not rely on animal data to determine the toxicity of a chemical or a threshold of toxicity. Rather the medical profession relies on human epidemiology data because they are more accurate than animal data. There are many differences in the response among species when exposed to a chemical and between strains within a species. Dr. Akhtar said her organization considers more favorably data generated in tests using human cells than data from tests using animal cells because tests using human cells are more likely to predict human outcomes.

   Dr. Moiz Momtaz asked Dr. Akhtar how confident she is that the epidemiology data she relies on are derived from exposure to a single chemical since most exposures are to mixtures. Dr. Akhtar replied that the epidemiology data aid in assessing exposure information. She said risk assessments should be based on data from methodologies that are the most accurate and the most predictive of human outcomes. She added that the Department of Health and Human Services (HHS) and the FDA have declared that 90-92% of animal data from pharmacological testing fail to predict human outcomes for safety and efficacy. She proposed that risk assessors re-evaluate animal data and put animal tests through the same validation process being required of non-animal *in vitro* methods. Dr. Momtaz asked Dr. Akhtar for a copy of the HHS report and she agreed to provide it to Dr. Freeman.

E. SACATM Comments on the Draft Five-Year Plan and Working Group Report

Dr. Marsman, a lead SACATM discussant, said the plan makes a noble attempt to address the advancement of alternative tests while continuing to protect human health and the environment. The working group's comments are consistent with his thoughts about the draft NICEATM-ICCVAM Five-Year Plan. He focused his comments on two key areas where improvement is needed: (1) the development of a transparent process for tracking milestones and communicating progress and (2) a heightened focus on the implementation of alternative assays into everyday use in research and testing. He addressed the questions posed to SACATM.

Question 1: Does the plan adequately address the two objectives laid out by Congress: (1) research, development, translation, and validation of new and revised non-animal and other alternative assays for integration into federal agency testing programs and (2) identification of areas of high priority for new and revised non-animal and alternative assays for replacement, reduction, and refinement (less pain and distress) of animal tests.

Dr. Marsman said previous ICCVAM activities have adequately addressed Objective 2 by creating a list of priority areas. However, there are a few areas that were under emphasized such as reproductive toxicity. ICCVAM needs to generate an endpoint-specific tracking process (e.g., a spreadsheet or "scorecard") that will inform the public about a test method's prioritization and potential impact on the 3Rs and to track its progress through the ICCVAM review process.

A rationale for prioritization is valuable, but it is more critical to comprehensively meet the challenge of Objective 1 at all stages from method development through implementation. ICCVAM must continue to play a key role in facilitating research and development activities for new alternatives. The plan is weakest in providing strategic guidance on the needs and expectations of U.S. regulatory agencies. This critical guidance is needed to provide focused, efficient direction for research and development and for implementation of alternative methods as useful tools for protecting human health and the environment.

Question 2: Does the plan clearly articulate and address the four challenges?

a) Identify priority areas and facilitate the activities associated with these areas.

Dr. Marsman said NICEATM and ICCVAM have identified key priority areas (ocular toxicity, acute toxicity, biologics, dermal toxicity, immunotoxicity, endocrine disruption, pyrogen testing, and chronic toxicity/carcinogenicity). However, there is little concerted effort to focus these priority areas towards the ultimate goal of providing validated methods for use by registrants and investigators.

b) Incorporate innovative research initiatives, which will lead to new alternative test methods.

Dr. Marsman said the working group clearly identified the unique position of NICEATM to provide scientific support for innovative alternative research and operational support for ICCVAM-related activities. ICCVAM should foster research at the 15 agencies and within academia and industry, which is distinct from its validation and implementation role.

c) *To encourage acceptance and approval of alternative test methods to reduce, replace, or refine the use of animals (including issues involving their pain and distress).*

Dr. Marsman said the plan lacks strategic depth in providing adequate guidance for the validation and integration of new methodologies into agency policy and expectations. The plan should be broadened to include specific feedback from the 15 ICCVAM agencies on specific needs for validation and integration to meet the agencies' and industry's obligations toward providing sound risk assessments for each endpoint. The ICCVAM process should be expanded to provide milestones of progress toward achieving the ultimate goal of implementing the alternative method. Progress toward these milestones could be recorded using the "scorecard." The process should define success criteria to meet each agency's needs for human health protection and for each assay/endpoint and also to capture global hurdles to test method implementation.

d) *To develop and strengthen both national and international partnerships to facilitate the progress of the activities addressed in (a) to (c).*

Dr. Marsman said the plan should clarify the distinctive roles of ICCVAM and NICEATM and provide a clear focus for ICCVAM's role in facilitating the integration of alternative methods into practice by the appropriate agency(s) and their regulated industrial sectors. The current ICCVAM working groups are useful, but only they provide preliminary feedback on the status of methods and recommendations for model refinements.

He concurred with the working group's recommendation that efforts should be made to incorporate stakeholder participation and feedback at each stage of development, validation, and integration of new methods in order to meet stated objectives and avoid redundancies. He noted that the complete replacement of animals in research and testing is clearly an onerous task. Stakeholder partnerships are critical. The ICCVAM working groups and SACATM should be better integrated, and ICCVAM should facilitate scientific meetings and workshops to (a) clarify the target, (b) identify gaps between human health needs and progress in animal alternatives, (c) encourage research and development of methods to address these gaps, and (d) monitor progress to ensure successful validation and integration of these methods into health risk and decisions.

Dr. Charles, a SACATM lead discussant, agreed with the findings of the working group regarding the lack of defined objectives and activities for ICCVAM to achieve the plan. He said the progress of the plan could not be evaluated over the next five years without defining the objectives and having a metric for measuring success. Presently, the plan describes a series of unfocused activities and there is no rationale for their prioritization. He agreed there should be better integration with stakeholders.

Dr. Dong, a SACATM lead discussant, had concern about the adequacy and appropriateness of the draft five-year plan. The draft attempts to justify the existence of ICCVAM by outlining some of its accomplishments over the past few years. Instead, the plan should identify the alternative tests that will be evaluated in the next five years. He pointed out that the draft does not include reproductive and developmental toxicity testing as a priority although this area of testing requires sacrificing far more animals than a single acute toxicity test. The plan should provide data or reasons for developing an alternative test and suggest a mechanism to save more animals per assay.

Dr. Bradlaw, a SACATM lead discussant, said the present plan is longer than requested by Congress and she hopes the shortened version will capture the important issues. She specifically identified Chapter three as needing improvement.

Dr. Ehrich said that the present version of the plan must be reduced to the requested 20 pages and tables should be included wherever possible to summarize the plan. Dr. Wind replied that there are a number of appendices, which are not counted in the page limit, but the present document is still too long.

Dr. McClellan said he had looked forward to reviewing the strategic plan because it provides an opportunity for ICCVAM to inform the Congress on its past, present, and future activities. Although the strategic plan was exceptionally well written, he had some concerns about it. First, the draft plan lacks a historical orientation and should describe where ICCVAM is today in the context of where it has been and where it is going. Second, it lacks any description of resources. He recognized that this requirement was imposed externally, but thought it would be impossible to evaluate the plan without some idea of the resources required for its implementation. Third, the draft plan lacks a management scheme and a description of how it will be implemented. He believed that ICCVAM's success has been limited by its *ad hoc* interagency approach, which is dependent on the goodwill of representatives of the different agencies. Historically, the responsibilities of the individual agencies have been ill defined and he applauded comments that call for more emphasis on timelines and explicit role(s) of the individual agencies. He mentioned the recent National Research Council (NRC) report, *Toxicity Testing in the 21 Century: a Vision and a Strategy*, but noted that the report is clearly deficient in not incorporating the type of studies being addressed in ICCVAM's five-year plan and suggested that the NRC report and ICCVAM's strategic plan should be linked. He urged that more attention be given to management and a paragraph be added about resources.

Dr. Barile commented on the lack of detail on developing partnerships and strengthening interactions with stakeholders. Part of fostering partnerships and creating collaborations is the funding of grants and providing opportunities for the research community to develop new tests, which helps ensure that the pipeline of new tests is ongoing.

Dr. Hayes thought the acute dermal test should have a high priority because the evaluation of tests in this area could be implemented immediately. He thought that communication with stakeholders important and that ICCVAM should be more proactive in seeking out useful tests that could be brought forward for validation. He noted that since NICEATM-ICCVAM reports are not published in the peer review literature, there is little awareness of their activities by the scientific and medical communications in general; he encouraged that this situation be remedied.

Dr. Stevens said the congressional mandate requested information on priorities. ICCVAM has interpreted this request by discussing priorities for specific endpoints such as acute toxicity and immunotoxicity. ICCVAM should present a strategic plan that describes what has transpired since its inception, what the obstacles have been, where ICCVAM is heading, and how it can improve its efficiency in accomplishing its goals. The plan should address procedural and technical issues. ICCVAM needs to consider whether the current validation process is too cumbersome and daunting for academic scientists. He noted that Dr. Willet raised the issue of using animal data as the reference standard because of its potential limitations in reliability and

relevance for humans and he suggested that ICCVAM should attempt to find reliable human data whenever possible.

Dr. Hartung, ECVAM liaison to SACATM, said ECVAM's Scientific Advisory Committee (ESAC) and ICCVAM have very similar mandates and there is considerable overlap in U.S. and European priorities, which should facilitate cooperation between the two groups. In the future, the efforts of the Japanese and Korean governmental groups should be added. He suggested developing a mechanism for formal interaction among the groups to share the burden of validation, rather than having a duplicative effort. ESAC and ICCVAM need to create harmonized peer review processes. OECD has laid the groundwork by creating Guidance Document 34 on validation. Common principles for validation are used worldwide, but there is no common mechanism to accomplish validation. He pointed out the first joint validation study by ICCVAM, ECVAM, and JaCVAM would evaluate the *in vivo* Comet assay for detecting genotoxic substances. He said it is remarkable what ICCVAM and NICEATM have achieved over the past 10 years with a limited budget; Europe invests 10 times more in *in vitro* tests. Dr. Hartung agreed with Dr. McClellan that a budget-neutral strategy is inadequate.

Dr. Stokes thanked the panel members for their thoughtful comments on the draft five-year plan. He asked ICCVAM members to respond to SACATM's suggestions regarding management and implementation plans. He asked the SACATM working group for suggestions of the two or three priorities to highlight and/or the criteria to use to identify the priorities.

Dr. Freeman said the five-year plan should discuss where ICCVAM wants to be in five years and what the committee wants to achieve in that time period-whether it is to, for example, validate specific tests, change the procedure for prioritization, or improve the validation process. The plan should list specific goals, describe how each goal will be accomplished, and identify the measures that will be used to monitor each goal's progress. ICCVAM must stress their historical successes and one way to do that would be to compare the original mission to what has been accomplished.

Dr. Hayes suggested that ICCVAM focus on dermal and ocular tests, which could be completed within a five-year period. ICCVAM could make a significant impact on reproductive tests because of the large number of animals that are needed, but such a goal would likely require a longer timeline to achieve.

Dr. DeGeorge suggested that milestones to achieve the development of reproductive tests would be useful for showing progress in that difficult area.

Dr. Becker said the plan stresses coordination and leverage outside the United States. He cautioned ICCVAM to stay focused on those activities that address the needs and desires of the ICCVAM agencies. ICCVAM should include activities that will be easily achievable within the five-year time frame and can be added to the regulatory testing framework. He thought that extensive research and development will be needed for reproduction and developmental tests; therefore, if this area is included as a priority, the focus should be on end points and tests where research and development are largely finished so that the final stages of validation and regulatory acceptance of the tests can be achieved in the near term.

Dr. Marsman agreed with Dr. Becker that the plan should be U.S.-focused, but the plan should also acknowledge the ongoing work worldwide. ICCVAM needs to pay greater attention to

implementation of validated methods that meet U.S. regulatory needs and provide that information to those scientists who need the methods for research or for estimating risk.

Dr. DeGeorge agreed that the United States should be able to benefit from what is done in Europe and stimulate collaboration among groups internationally; a special committee should be set-up to enhance this collaboration. There needs to be more discussion on collaboration and cooperation in the five-year plan and one of the appendices should contain information about how the United States can interact with other countries.

Dr. Becker agreed that enhancing collaboration and cooperation is critically important, but the plan must document the needs and priorities of U.S. regulatory agencies. He was concerned that if too much attention and resources are devoted to tracking and collaborating, the United States may miss opportunities to develop methods that can be utilized for federal regulatory testing programs. The regulatory structures and risk assessment methodologies in the United States may not be the same in other countries. Therefore, it is incumbent upon U.S. federal agencies to exert the leadership needed to assure that those methods, which are critically important, have an opportunity to be validated within a reasonable period of time.

Dr. Stephens said one approach to international cooperation might be to include a criterion that evaluates the impact or value of ICCVAM's participation with ECVAM and the other international agencies. Dr. Becker agreed with Dr. Stephens, but reiterated that the plan needs to describe how an activity will impact, influence, and/or involve U.S. regulatory agencies. If a linkage can be demonstrated between an assay that is necessary for a U.S. federal agency and its validation internationally, that should also be considered.

Dr. Freeman said he agreed with the international discussion. The reduction or replacement of animals in testing should be an international effort and, thus, cooperation and collaboration should be a major goal. He thought it imperative "to share the burden as Dr. Hartung suggested since it is a global marketplace.

Dr. Marsman said the plan should describe the state of research and development or pre-validation for each method and end point or whether the state of the science for an endpoint is at a stage where the method could be validated. If the method is at the research and development stage, the plan should indicate whether the work is being done in industry or through ICCVAM. If the method is at a monitoring stage, ICCVAM needs to facilitate its development into a usable and useful assay.

Dr. Freeman said there seemed to be strong consensus among SACATM members that the draft five-year plan needed more detailed, transparent plans, emphasis on historical successes, and details about resources. SACATM's views seemed consistent with the working group and the public comments. SACATM also discussed procedural issues, test priorities, international cooperation, and harmonization of processes.

Dr. Freeman called for a motion from SACATM to accept the working group's report on the five-year plan. Dr. Barrile made a motion to accept the report and Dr. Bradlaw seconded the motion.

Dr. Wolfe said SACATM could accept the working group report as written, reject the report, or amend the report. She added that the discussion at today's meeting would be captured in the minutes and would supplement the report. Following some discussion, Dr. Barile suggested that SACATM vote on acceptance of the working group report and then supplement it with SACATM's comments. Dr. Diggs concurred with Dr. Barile.

Dr. Freeman asked Drs. McClellan and Marsman their thoughts on Dr. Barile's proposal. Dr. McClellan suggested that a sentence to be added to the working group report that referenced the additional discussion at the SACATM meeting and the minutes.

Dr. Wolfe said the text could be added to the working group report administratively. Dr. Becker asked Dr. Wolfe if it would be acceptable for the four lead discussants to add any materials to the working group report as an appendix, and Dr. Wolfe responded that SACATM would need to agree to the addition.

Dr. Stephens recognized the time and valuable input provided by the four SACATM lead discussant and noted it would be important include their materials. Dr. Freeman suggested that the working group report be appended to the meeting minutes. The minutes could include the comments by the lead discussants and the general SACATM discussion.

Dr. Freeman asked for a motion. Dr. Barile moved that SACATM accept the working group report as written adding that reference to the minutes in the working group report and to the comments by the lead discussants and SACATM in general in the minutes be incorporated as Dr. Wolfe deemed appropriate. Dr. Bradlaw seconded the motion. SACATM accepted the motion unanimously with 12 yes votes, 0 no votes, and 0 abstentions. Dr. Becker noted it important that the working group report and the minutes with the discussion be linked as a stand-alone document on the meeting website, so future readers are fully informed about the breadth of the dialogue.

[Note: the working group report was edited to contain text referring the reader to the SACATM minutes for this meeting. A summary of the discussion by the lead discussants and SACATM in general is included in the minutes, and the working group report is attached to the minutes as Appendix 1. Both are posted on the SACATM website for this meeting.]

VI. Overview of the ICCVAM Evaluation of *In Vitro* Pyrogen Test Methods

A. Presentation

Dr. Richard McFarland, U.S. Food and Drug Administration (FDA), ICCVAM Pyrogenicity Working Group (PWG) Chair, presented an update on ICCVAM's ongoing evaluation of five *in vitro* human cell-based pyrogen test methods nominated for review by the European Centre for the Validation of Alternative Methods (ECVAM). Pyrogenicity is defined as an increase in body temperature following the release of pro-inflammatory cytokines [e.g., interleukin (IL)-1, IL-6, and tumor necrosis factor-α (TNF-α)] by leukocytes. Pyrogens may be found in processing and packaging materials, chemicals, or parenteral pharmaceuticals, biologicals, and medical devices. Bacterial endotoxin, a component of the outer cell wall of Gram-negative bacteria, is one of the

most potent pyrogenic materials. Pyrogen testing is important to prevent the introduction of endotoxin or non-endotoxin pyrogen-contaminated products into humans or animals.

Currently there are two accepted pyrogen tests. The Rabbit Pyrogen Test (RPT), which measures a temperature rise in rabbits injected with a test substance, can detect both endotoxin and non-endotoxin pyrogens. The Bacterial Endotoxin Test (BET), also referred to as the *Limulus* Amoebocyte Lysate (LAL) Test, detects endotoxin by its ability to activate a serine-protease catalytic cascade.

In June 2005, ECVAM submitted background review documents (BRDs) on five methods for consideration by NICEATM as replacements for the RPT. The methods are:

- Human Whole Blood (WB)/Interleukin (IL)-1 *In Vitro* Pyrogen Test
- Human WB/IL-1 *In Vitro* Pyrogen Test: Application of Cryopreserved Human WB
- Human WB/IL-6 *In Vitro* Pyrogen Test
- Human Peripheral Blood Mononuclear Cell (PBMC)/IL-6 *In Vitro* Pyrogen Test
- *In Vitro* Pyrogen Test using the monocytoid cell line, Mono Mac 6 (MM6)/IL-6

Before describing the evaluation process, Dr. McFarland listed the members of the PWG, provided a time line for the various activities connected with the evaluation process, and described the ICCVAM acceptance and validation criteria for alternative test methods.

Following a prescreen evaluation, NICEATM requested additional information and clarification from ECVAM in regard to the data provided in their BRDs. ECVAM submitted revised BRDs that addressed these requests. Subsequently, ICCVAM prepared a draft ICCVAM BRD that contained a comprehensive review of all available data and information regarding the usefulness and limitations of the five alternative *in vitro* pyrogen test methods and described the current validation status of the test methods including their relevance, reliability, scope of substances tested, and the availability of a standardized test method protocol for each test method.

The major difference among the five test methods is the cell types used; the methodology used for the test methods is very similar. Briefly, the test substance is applied to cultures of the specific human-derived cells, which are then incubated for 16-24 hr. The concentration of pro-inflammatory cytokines (e.g., IL-1β, IL-6) is quantified via a cytokine-specific enzyme-linked immunosorbent assay (ELISA). The endotoxin activity of a test substance is calculated by comparing the induced cytokine release with that induced by the endotoxin standard.

The test methods were reviewed for their ability to detect the presence of Gram-negative endotoxin when several parenteral pharmaceuticals were spiked with the endotoxin standard at several different concentrations. The reference pharmaceuticals were considered positive for endotoxin if the endotoxin content was > 0.5 endotoxin units (EU)/mL. Differences were found in the performance of the five test methods. Based on the information contained in the BRD, ICCVAM developed draft recommendations for the use, formulated draft performance standards and draft test method protocols for each test method, and identified proposed future studies.

ICCVAM's draft recommendations on test method uses and limitations was that, based on the validation studies with a limited number of pharmaceuticals, there is sufficient information to

substantiate the use of these test methods for the detection of pyrogenicity mediated by Gram-negative endotoxins in materials that are currently tested in the RPT, subject to product-specific validation to demonstrate equivalency. Further, ICCVAM's draft recommendations stated that although the five *in vitro* test methods may be capable of detecting a wider range of pyrogens than were tested, the data in the BRDs do not support this broader application. One limitation of the validation study was the lack of a direct comparison of the results for the same test substances in the proposed *in vitro* test methods versus the RPT.

ICCVAM also provided draft recommendations for performance standards for these five *in vitro* test methods for consideration by the peer review panel and for public comment; the purpose of performance standards are to ensure that any proposed mechanistically and functionally similar proposed test method meets acceptable standards. Performance standards include essential test method components based upon common structural, functional, and procedural elements that should be included in the protocol of a mechanistically and functionally similar proposed test method; recommended reference substances for evaluating the relevance and reliability of the proposed test method and the performance characteristics (relevance and reliability values) that should be met or exceeded. ICCVAM also recommended draft standardized protocols that were based on those used in the ECVAM validation study. Finally, ICCVAM recommended future studies that included the testing of a broader range of pyrogenic materials under conditions where the *in vitro* pyrogen test(s) and the RPT were run in parallel to be able to directly compare the results.

B. Peer Panel Report

ICCVAM and NICEATM held a peer review panel meeting on February 6, 2007, to review the five *in vitro* pyrogenicity test methods. Dr. Karen Brown, DRL Pharma and Pair O'Docs Enterprises, chair of the peer panel, said the task was daunting because the panel was tasked to complete the evaluation of the five *in vitro* test methods in one day. She recognized the hard work and diligence of the panel.

The charge to the peer review panel was to review the draft BRDs for completeness, assess whether each applicable criterion for validation and acceptance of the test method had been appropriately addressed, and consider whether the information in the BRD supported the draft ICCVAM recommendations for the draft standardized protocols, the draft test method performance standards, and the draft proposed future studies.

The panel concluded that the explanation in the BRD of the usefulness and limitations of the *in vitro* pyrogenicity test methods and of the description of the current validation status of these methods was sufficient. However, they identified a number of deficiencies in the BRD, which are briefly described below.

1. There were some sections where additional details would have improved the document. For example, the panel wanted information included about (1) the number of RPTs conducted per year to evaluate bacterial endotoxin, (2) the number of rabbits used for *pyrogen*icity testing per year, and (3) the costs and logistical considerations for either setting up the cell culture for the MM6 test or obtaining human blood for the other tests.

2. The rationale for selecting the test substances for evaluating the five *in vitro* test methods was flawed because it did not represent the range of products tested for bacterial endotoxin using the RPT and seven of the 10 substances were not tested in the RPT but rather in the BET. For example, no biologicals or medical devices were evaluated. The panel felt that the number of substances tested in the validation study was not adequate to evaluate whether a specific test method could replace the RPT.

3. The *in vivo* RPT reference data were limited to one strain of rabbit tested in one laboratory by one protocol using two sources of bacterial endotoxin.

4. The evaluation of the relevance of each test method was adequately demonstrated and discussed in the BRDs, but was limited by the ability to judge a positive versus a negative response based on 0.5 endotoxin units (EU)/ mL. Since samples were only spiked with bacterial endotoxin, the relevance was only demonstrated for the detection of this type of pyrogen, and there was no evaluation for the ability to detect non-endotoxin pyrogens.

5. The discussion on concordance in the RPT is speculative because there was no parallel testing with the RPT, and the RPT performance was modeled statistically.

6. The whole blood IL-1 test is inadequate because there were too many false positives and false negatives; however the IL-6 assay appeared to perform better. It would have been more appropriate to compare these *in vitro* tests directly with the BET, since only bacterial endotoxin samples were used.

7. Test method reliability was acceptable in both within and between laboratory studies; however, a quantitative assessment of intra- and inter-laboratory variability would have been more informative. A statistical assessment providing acceptability criteria should have been performed to test the hypothesis that there were no differences among groups.

8. The assessment of test method reliability had the following deficiencies:

    - There was a high exclusion rate for individual runs of the whole blood IL-1 assay due to excessive variability among the four replicates.
    - The agreement across three validation laboratories was only 57% for the whole blood IL-1 assay.
    - The same subset of drugs tested for sensitivity and specificity should have been tested for reliability.

Most of the panel agreed that application of the validation criteria to determine the usefulness and limitations of these test methods to replace the RPT under conditions where the test was for the presence of Gram-negative endotoxin was adequately addressed in the BRDs.

The panel concluded that the usefulness of the test methods to detect Gram-negative endotoxin was not assessed properly to determine their concordance with the RPT or to compare their relevance with the BET. The assessment of the usefulness was limited because non-endotoxin

pyrogens were not included, and the pure form of the test materials may stimulate cytokine production.

The panel agreed that the BRDs did support the proposed standardized test method protocols if the list of its inadequacies were fully addressed. The panel noted that to reduce variability, similar acceptance criteria must be used for multiple blood donors and similar exclusion rules must be used for each test method. They recommended that a more specific protocol be developed that details recruitment of human blood donors, selection criteria for donors, as well as conditions for veinipuncture.

The panel concluded that the test method performance standards were not supported by the BRD. Statements about the five methods' accuracy and reliability were not supported because two assays demonstrated false-positive results greater than 16 % and the *in vitro* test methods should have been compared to both the BET and RPT. Also, the panel thought that the small list of substances was inadequate to assess whether these test methods could replace the RPT. Test substances need to include all classes of endotoxins as well as non-endotoxin pyrogens.

The panel agreed that additional studies should be performed, and that ICCVAM should consider their comments and recommendations. They suggested (1) establishment of a repository of clinically identified pyrogens to use in future validation studies, (2) inclusion of both endotoxin and non-endotoxin pyrogens in future validation studies, (3) prospective comparison of any *in vitro* tests with the RPT and BET, and (4) evaluation of IL-1 and IL-6 levels in the *in vitro* tests and their correlation with levels produced in rabbits exposed to similar levels of endotoxin. Overall, the peer review panel concluded that these five test methods could be applicable for a wider range of pyrogens and test materials if they were adequately validated for such uses. It is important to recognize that, despite the panel's concerns about the performance of these five *in vitro* test methods, the FDA has a formal process for materials regulated under 21CFR610.9 (e.g., parenteral drugs) that allows drug manufacturers to qualify *in vitro test* methods for identifying Gram-negative endotoxin, on a case-by-case basis.

C. Public Comments

1. Ms. Kristy Stoick, PCRM, said her organization submitted written comments after the peer review panel meeting. PCRM was disappointed with the ICCVAM draft recommendations and the peer review panel report. Since federal regulations specify that these methods must undergo product specific validation for pyrogenicity, she encouraged SACATM to recommend that ICCVAM help facilitate further development of these methods by companies so the regulatory community can begin to use them as soon as possible. She did not support additional *in vivo* validation studies.

2. Dr. Thomas Hartung, ECVAM, joined the public for this specific agenda item because of a conflict of interest as a patent holder for the methods. Three of the *in vitro* test methods were based on his research and he had coordinated the validation study prior to joining ECVAM. He was pleased that the European Pharmacoepia will hold a peer review panel to review and accept these methods. He was disappointed with the outcome of the peer review panel meeting. He noted that pyrogenicity tests are very expensive and the approval and release of

a single product can cost several hundred thousand dollars. The validation studies were set up to assess whether the new tests would outperform the old tests within a set threshold. Only 50% of the samples would be positive in the most sensitive rabbit strain. All of the *in vitro* assays have an accuracy of around 90%. He outlined six points where the BRD had been criticized.

ICCVAM said the BRD is deficient due to the limited data for only 10 pharmaceutical substances from the validation studies, which alone cost $6M. The recommendations for additional studies from the peer review panel would cost between $20-40M and they would be a waste of resources because a product-specific validation process would be required for each application. To help contain cost, the tests described in the BRD were designed to emphasize the accuracy of the method to detect pyrogens near the threshold.

The peer review panel did not acknowledge the difference in status of the five methodologies. Some methods are used in more than 80 laboratories while others are used infrequently; however, the same criticisms were applied to all of the methods.

The BRD recommended that parallel testing be conducted with the RPT. However, parallel testing in rabbits is unnecessary because these studies have been performed for 65 years using a WHO standard as a reference material. The outcome from rabbit testing is so predictable that ethically it is not warranted. Also, in the European Union, it will be impossible for ECVAM to carry out these *in vivo* tests especially as the new methodologies have shown partial concordance.

Endotoxins are tested only in the BET assay, and this assay has replaced the RPT for about 90% of substances; the remaining 10% of substances consist of non-endotoxin pyrogen products that interfere with the BET. He asked why the new tests have to meet higher standards than the BET, which has been endorsed for the testing of many pyrogenic products. He noted that no reference non-endotoxin pyrogens are suitable for validation purposes in rabbits and humans; therefore, inclusion of such controls is scientifically impossible.

High endotoxin concentrations will be detected accurately in the RPT, BET, or any of the new *in vitro* pyrogenicity assays. Hence, a concentration near 50 pg of endotoxin, which is equivalent to 0.5 EU and is the threshold for rabbits, was chosen for the assays. Additional concentrations of 100 pg and 25 pg were also selected. Even though the assays were challenged at these low concentrations, they were 90% accurate. False positives were due to spikes at half the threshold indicating that the assays are too sensitive.

The new assays were evaluated fairly in comparison to the limitations of the existing tests. The rabbit test, which has a number of limitations, has never been properly validated for non-endotoxin pyrogens. The BET does not detect all Gram-positive endotoxins although the new assays have shown some capability for doing so.

In conclusion, the proposed test methods for which data sets have been provided perform better than the BET and RPT. Dr. Hartung proposed that the rabbit assay be replaced with the *in vitro* assays because the RPT cannot match their performance, as reported in the BRD.

D. SACATM Discussion

SACATM was asked to address questions regarding the peer review panel's conclusion and recommendations of the draft ICCVAM BRD with regard to its completeness; the panel's identification of errors or omissions; whether ICCVAM's applicable criteria for validation and acceptance of toxicological test methods were addressed; and to provide comments on the draft ICCVAM test methods recommendations, usefulness of the test methods, the test method protocols, proposed performance standards, as well as proposed additional studies.

Dr. Barile, a lead discussant, said there was no question about the usefulness of pyrogenicity testing and the urgency and importance of validating these tests. In combination, some of these tests will contribute to the reduction of animal usage. One major deficiency of present pyrogenicity testing is that the RPT only detects about 50% of the endotoxins. Some of the proposed *in vitro* tests had false negative responses in the range of 10% while the IL-1 assay had a false negative response of 27%. These false negative responses could be due to consistently higher variability among some donors, which would be a limitation relative to a whole blood human assay. He expressed concern that the IL6 ELISA test, marketed by Novartis, is a proprietary test and he would not recommend approving a method without knowing the experimental details. He agreed with Dr. Hartung that parallel testing in rabbits was unnecessary during development of the testing methodologies. However, a comparison to RPT data is necessary so that a valid concordance or regression analysis between the *in vivo* and *in vitro* methods can be undertaken. He said samples spiked with endotoxin are not representative of real world samples such as a biological vaccine or a solubilized pharmaceutical product. There is no solubility problem associated with the testing of biological vaccines in rabbits, but insolubility is a problem in *in vitro* tests even if the test article is in suspension and this technicality must be addressed. He believes that the cell culture methods are more developed than the whole blood methods for validation purposes. A few additional studies, which address the panel's recommendations, would allow the cell culture pyrogenicity tests to receive validation status.

Dr. McClellan said he was generally pleased with the draft BRD until he heard Dr. Hartung's statement. He did not believe that the BRD is adequate nor can he compliment the peer review panel on its report. He wondered how this difference of opinion would be resolved and asked Dr. Brown to comment.

Dr. Freeman said he was confident that all of SACATM's comments would be taken into account by ICCVAM and, if necessary, ICCVAM could reconvene the expert panel.

Dr. Brown said ECVAM produced a reasonably comprehensive BRD, but the panel was not able to address all of the components of the individual *in vitro* methods because time for discussion was limited. Some of the details were missing or difficult to understand; however, she felt that given more time to discuss these methods, the panel might have been able to provide a stronger recommendation for one or more of the assays. Personally, she felt that the MM6 assay has the greatest potential and several of the other panel members agreed. The most bothersome aspect for the panel was trying to identify the specifics of the validation protocols. She noted that for an *in vitro* assay it is critical to identify every component and every single condition of the assay

completely, but this information was not provided, particularly for the MM6 test method. She was impressed with the cell culture methodology, although specifics such as cell passage levels, or how many cells are used in a test were lacking. She felt that the panel did not seem to understand cell culture methodology and its related costs. Consequently, they got side-tracked in specifics, which hindered them from making progress and reaching conclusions.

Dr. Brown said she does not believe that it is necessary to run *in vivo* assays in parallel with the *in vitro* assays. She is unsure how one can run a regression analysis with one test that is 90% accurate and a second that is 50% accurate. She questioned whether it is necessary to validate an *in vitro* test against an animal test that is not as accurate as the *in vitro* assay itself.

Dr. McClellan said Dr. Hartung disclosed his own potential biases, concerns, and background. He asked whether Dr Hartung was suggesting that two of the assays should have received more attention and wondered which of the assays Dr. Hartung thought were appropriately validated and whether he might focus the panel toward those assays.

Dr. Stokes said in the future NICEATM would set aside at least two days for a peer review meeting, so that a panel can fully understand the methodologies before they deliberate on the evaluation questions.

Dr. Qu had some comments on the panel's concern about data transformations. The panel was not sure if the data were transformed and whether or not the use of a "t" test for their analysis was appropriate. She said it is not necessary to use a "t" test even if the data are normal. A non-parametric test such as the permutation test, which does not require transformation, could be used. Dr. Qu noted also that it is important to control for false positives when doing a multiple comparison for several tests. By doing multiple comparisons, it is possible to obtain a statistically significant difference that is not biologically significant. One approach to dealing with this problem is to use a more stringent level of significance.

Dr. Becker welcomed the proposed longer time frame for a peer review meeting. He suggested that it might be useful to convene a meeting with a core panel of validation experts and then have subject-specific experts to address specific assays.

VII. Local Lymph Node Assay Nominations

A. Presentation

Dr. Raymond Tice, NICEATM, discussed the ICCVAM nominations for the local lymph node assay (LLNA). He noted that SACATM's recommendations would be presented at an upcoming ICCVAM meeting where ICCVAM would consider the priorities for the LLNA activities.

Dr. Tice explained that skin sensitizers induce lymphocyte proliferation in the lymph nodes that drain the site of application and that the increase in lymphocyte proliferation in treated versus control nodes is used to identify chemical sensitizers. When ICCVAM reviewed the LLNA in 1998, the peer review panel concluded that it was validated sufficiently as a stand-alone alternative test to guinea pig test methods for identifying skin sensitizers, but noted that data on

the testing of metals was limited. Based on the ICCVAM evaluation and recommendations, the LLNA protocol was incorporated into the EPA Testing Guidelines for Skin Sensitization and the OECD testing guidelines (TG 429) for the assessment of skin sensitization as an alternative test method.

CPSC nominated the LLNA for further study to increase its applicability. The nomination asked ICCVAM to address (1) whether the LLNA could be used as a stand-alone assay for determining potency for hazard classification, (2) whether non-radioactive reagents could be used in LLNA protocols, (3) whether a "cut-down" or "limit" test would be appropriate, and (4) whether the LLNA could be adapted to test mixtures, aqueous solutions, and metals. A "cut-down" or "limit" test would allow the use of one dose instead of three and would thus reduce the number of animals per test by at least 40%. ECVAM's ESAC has approved the LLNA limit test for hazard classification.

Dr. Tice said several regulatory agencies evaluate hazards from skin sensitization for labeling purposes. The "limit" test and the other nominated activities would reduce and refine animal use compared to the guinea pig tests, which are associated with some animal pain and suffering, use more animals, and take longer to perform. These adaptations would also increase the potential for the LLNA to measure potency and expand its use because laboratories would not be required to handle radioactivity.

NICEATM published a *Federal Register* notice on May 17, 2007, inviting public comment on the appropriateness and relative priority of related activities including preparing background review documents, developing performance standards, and convening an expert panel to review the nominated methods. In addition, NICEATM invited the nomination of expert scientists to serve on a peer review panel, if convened, and the submission of data from traditional and/or modified versions of the LLNA.

Planned NICEATM activities include: (1) searching for relevant data and information from the published literature and by contacting interested stakeholders, (2) expanding the 1998 database of results for test substances, (3) re-evaluating LLNA performance compared to the guinea pig test methods and data from humans, (4) preparing draft background review documents that provide a comprehensive review of the validation status of the various methods, and (5) drafting ICCVAM test method recommendations. Once completed, NICEATM will convene an expert panel to peer review the draft background review documents and the draft test method recommendations.

Dr. Tice outlined the proposed timeline for this nomination; the peer review is tentatively planned for February 2008. NICEATM has received three public comments that support the nominated activities, provide data, and nominate experts for the panel.

B. Public comments

1. Dr. Melissa Kirk, MB Research Laboratories, said her company supports CPSC's request for NICEATM and ICCVAM to evaluate non-radiolabeled versions of the LLNA test method and offered its assistance in validating these methods. The company has developed a flow

cytometric-based LLNA method termed FCLLNA to assess acute dermal sensitivity. The FCLLNA assesses proliferation by evaluating lymph node cell number and determining incorporation of the thymidine analogue bromodeoxyuridine into the DNA of lymph node cells. This assay is safer to conduct because it eliminates use of hazardous radioactive material. The FCLLNA is similar to the ICCVAM-validated LLNA protocol in the dosing method, assay schedule, vehicles, and positive control. A stimulation index equal to 3 indicates a positive response to sensitization in both the validated and FCLLNA protocols. LLNA and FCLLNA differ as the FCLLNA uses a multifactorial approach to evaluate sensitizers and eliminate false positive irritants. True sensitizers are determined by evaluating stimulation index, ear swelling measurements, and phenotypic markers. A major advantage of the FCLLNA is that cell surface markers can be analyzed on cells harvested to determine the stimulation index so no additional animals are needed. The FCLLNA demonstrated stimulation indices similar to those reported in the ICCVAM radiolabeled validation study when over 50 chemicals, including sensitizers, non-sensitizers, and irritants, were tested. The FCLLNA has 95% accuracy compared with the radiolabeled assay. The FCLLNA is less accurate than the radiolabeled LLNA when compared to guinea pig tests; however, it is more accurate than the LLNA when compared to human tests. MBRL has performed more than 80 FCLLNA assays for clients for safety evaluations and for submission to regulatory agencies. Augmentation of the original LLNA with flow cytometry end points increases the sensitivity and discriminatory power of the LLNA while reducing the number of animals needed. It also increases the quality and quantity of data generated when compared with existing methods and substantially reduces the assay-cost because it does not use radioactivity. MBRL proposes to submit the FCLLNA protocol and validation data to ICCVAM for consideration as a direct substitute to the guinea pig sensitization tests. It is hoped that MBRL's submitted data will be sufficient to validate this modification.

2. Dr. Catherine Willett, representing People of the Ethical Treatment of Animals (PETA), said the majority of the activities in CPSC's request have been completed, so PETA is hopeful that NICEATM and ICCVAM will rapidly expedite any additional studies. The European Center for Ecotoxicology of Chemicals published a comprehensive review of sensitization test methods and concluded that the LLNA is a viable and complete alternative to the traditional guinea pig tests for the purposes of skin sensitization, hazard identification, skin sensitizing potency, and derivation of EC3 values [i.e., the concentration of a test substance that induces a 3-fold increase in the stimulation index (SI); an SI of at least 3.0 is the decision criteria used to classify a substance as a sensitizer], which provide a quantitative measure of potency. The report further states that the LLNA is the recommended method for new assessments of relative potency and for investigating the influence of vehicle or formulation on skin sensitization potency. Recent work has verified that there is a clear, linear relationship between LLNA-derived EC3 values and historical skin-patch data. A retrospective analysis published in 2006 of the regulatory use of the LLNA in the European Union concluded that the LLNA is satisfactory for routine regulatory use.

   She pointed out that ESAC recently issued a statement supporting the use of the "reduced LLNA," to reliably distinguish between chemicals that are skin sensitizers and non-sensitizers, which could reduce animal usage by 50%. One caveat, however, is that this

method cannot be used to test the potency of sensitizing chemicals at concentrations less that 10%.

PETA feels that another lengthy, detailed review of the LLNA is not warranted because the method has been used by regulatory agencies for classifying skin sensitizers for years and both research and regulatory use of the LLNA has been extensively reviewed in the literature; PETA supports an expedited review process. PETA hopes ICCVAM will promote the development and regulatory use of non-animal methods through participation in integrated groups such as the European Sensitive Project that brings together regulatory agencies, industry, and academia. She pointed out that additional non-animal methods for estimating sensitivity are under development. Several QSAR modeling projects show very high concordance with both guinea pig and LLNA data, and methods that use human cell culture technologies to quantify peptide reactivity show very high concordance with LLNA data. Dr. Willett encouraged ICCVAM to apply its resources toward validation and regulatory acceptance of one or more of these methods.

Dr. Ehrich asked if Dr. Willet were recommending that the background review documents be updated without any further testing and Dr. Willet responded yes, that she understood the objective for this LLNA nomination is to review existing material. Further, scientists from all over the world have already reviewed these assays; therefore, ICCVAM should move forward and build on assessments that have already been carried out and apply them to the specific U.S. needs.

Dr. Becker asked Dr. Willett's opinion whether a peer review group should contribute to the development of performance standards and Dr. Willett replied no, but added that previous reviews of existing data on these various modifications of the LLNA should be sufficient to determine if they are appropriate for regulatory needs.

In response to a question from Dr. Becker, Dr. Wind said the Global Harmonization System group is considering changing the classification system for sensitization to differentiate potency between two different classes "severe" and "weak"; now potency is classified simply as "yes" or "no".

Dr. Becker asked Dr. Tice to explain performance standards and why they are needed for the LLNA. Dr. Tice said performance standards could be used for accelerating the development of alternative test methods by providing standards for a specific existing test method against which to compare the performance of a new test that is functionally and mechanistically similar. For example, normally a company wanting to validate a variation of the existing test method would have to conduct a complete validation study. However, if the test developer can show that the variation meets the performance standards for the existing method, then the validation study would not be necessary. Performance standards for the LLNA would also aid in the development of *in vitro* alternatives for skin sensitization.

3. Ms. Kristine Stoick representing the Physicians Committee for Responsible Medicine (PCRM) thanked the CPSC for its nomination. Her organization is always interested in making current tests better provided that the highest priority is given to reducing animal use.

The PCRM believes the "cut-down" or "limit dose" approach is important since this non-radiolabeled LLNA method would result in a reduction of animals. She agreed with Dr. Willett on an expedited scientific review and encouraged ICCVAM to work with scientists and regulatory authorities to facilitate the use of non-animal methods for the sensitization end point. She encouraged ICCVAM to focus on appropriate nonanimal methods, such as those presented at the immunotoxicology meeting in Brussels last year, instead of spending resources on expensive evaluations of the LLNA, because it is an animal test.

C. SACATM Discussion

SACATM was asked to comment on (1) whether ICCVAM should validate the LLNA as a stand alone test for determining potency to classify hazards, (2) whether the non-radioactive modification should be reviewed, (3) whether the LLNA could be modified so that the "cut down" or "limit" procedure can be used and (4) whether the LLNA is sensitive to mixtures and metals.

Dr. Freeman said SACATM would vote whether to accept the high priority nomination to prepare a comprehensive BRD and conduct a review of these new approaches to the LLNA.

Dr. Cunningham said that her comments encapsulated those from Dr. Diggs, Dr. Ehrich, and herself. The LLNA was accepted eight years ago as an alternative to the guinea pig maximization or Buhler assay with limited use for hazard identification. The CPSC nomination would broaden the scope of the LLNA to include potency and severity determinations, reduce animal use through the "limit dose" protocol, refine the assay by a non radioactive modification, and broaden the types substances it could test.

Dr. Cunningham raised questions encountered during her group's review namely (1) whether the potency and severity would be determined by using the EC3 concentration in the LLNA, (2) whether there is an overlap with the plans of ECVAM and JACVAM that would provide an opportunity for a partnership, and (3) whether substances not previously tested would be included in the list of aqueous solutions, mixtures and metals. The documents provided by CPCS discussed other options such as QSAR's and single vs. repeated dosing, but it was not clear why these items were selected for validation. The group questioned whether a validation study is needed for ICCVAM to make a recommendation on this nomination or are there other approaches that might be undertaken.

Dr. Wind clarified that CPSC is not requesting a validation study. She said ICCVAM would be evaluating data published since the last review of the LLNA, when the LLNA was recommended as a stand-alone assay for a "yes" or "no" response. At that time, there were limited data on the testing of mixtures, aqueous solutions and metals. CPSC is evaluating EC3 test results and comparing them to guinea pig and human data to determine if the EC3 actually predicts differences in potency. Non-radioactive methods are being used in countries or by organizations where radioactive methods are not allowed, despite the fact they have not been validated. U.S. regulatory agencies can only accept data from a validated method. The ICCVAM Immunotoxicity Working Group includes representatives from ECVAM and JaCVAM. In answer to a question from the audience, Dr. Wind said it was clear at the Berlin meeting that the

QSAR approach is very limited, and thus the working group felt that it would be counter productive to pursue it.

Dr. Becker thought that it was critical in going forward with this nomination that performance standards be developed and peer reviewed. Performance standards should be considered every time a new method is proposed for review because they provide a benchmark to judge the applicability of an alternative method. This approach may be a way to bring alternative methods forward more expeditiously provided they are shown to perform according to the performance standards.

Dr. Diggs questioned whether carrying out this nomination is the best use of resources and asked about the potential benefit for reducing animal use. Dr. Stokes said the LLNA is one of the six most commonly conducted consumer product safety tests. He did not know how many guinea pig tests are still conducted vs. the LLNA, but believed it to be fairly significant. This activity would move the LLNA into the mainstream where it can become a replacement rather than just a substitute test as it is now. Dr. Wind said the EPA Office of Pesticides tells applicants not to submit LLNA data on mixtures, aqueous solutions, or metals since it has not been validated for those types of substances.

Dr. Freeman called for a motion from SACATM on the LLNA nomination as a high priority. Dr. Cunningham moved to accept the nomination and Dr. Barile seconded the motion. SACATM voted 12 yes, 1 no, 0 abstentions in favor of the nomination. Dr. Dong said he voted against the motion because he had no data that there would be an impact on guinea pig use and he was unsure how undertaking this nomination might impact future ICCVAM activities.

Dr. Stokes replied that presently two guinea pig assays, the guinea pig maximization assay and the Buhler assay, are used. The LLNA assay uses fewer animals than the guinea pig-based test and eliminates the pain and distress associated with a positive result in guinea pigs or when complete Freund's adjuvant is used to help stimulate a response. He said the LLNA uses 20 animals per test vs. 28 or more animals for the guinea pig test, so the LLNA would save about 10 animals per assay. He estimated that several thousand new products are tested each year for an estimated saving of about 10,000 guinea pigs. Dr. Stokes also said that currently this nomination could be accommodated without having an impact on future activities.

Hearing these remarks, Dr. Dong said he could support the nomination if pursuing these LLNA activities would not slow down ICCVAM's progress over the next five years in carrying out the 5-year plan. Dr. Stokes said it would not.

VIII. High Throughput Screening (HTS) Initiative

A. Presentation

Dr. Tice said a major component of the NTP Roadmap for the 21st Century is to develop HTS assays that could be used to: (1) prioritize chemicals for further in-depth toxicological evaluation, (2) identify mechanisms of action, and (3) develop predictive models for *in vivo* biological responses. He briefly described the NTP HTS Screening Assays Workshop that took

place on December 14-15, 2005, in Arlington, VA that included participants from industry, academia and the government. There were four breakout groups: (1) Selection of Targets and Assays for HTS, (2) Chemical Selection, (3) Study Design and Analytical Methods, and (4) Data Storage, Analysis, and Interpretation.

He noted that the workshop's participants thought a near-term goal would be to use HTS assays for priority setting, but their use in regulatory decision-making would be a longer-term goal. Appropriate training of regulatory scientists on the use and interpretation of data from these assays would be needed.

In mid-2005, the NTP became a formal participant in the NIH Molecular Libraries Initiative (MLI) by establishing collaboration with the NIH Chemical Genomics Center (NCGC). Using robotics, the NCGC has the ability to test several hundred thousand compounds in one assay per week. This interaction with the NCGC will provide an opportunity for NTP to link data generated from HTS assays for biological activity with toxicity data from NTP's testing program. Currently, the NCGC is testing 1408 compounds provided by the NTP in a series of *in vitro* cell- or biochemical-based assays. Of the compounds submitted, 1353 were unique and 55 were duplicates to evaluate assay reproducibility. NTP has toxicologic data on 1206 compounds and 147 are ICCVAM reference substances for the validation of alternative *in vitro* test methods (e.g., dermal corrosion, acute toxicity, endocrine activity). Selection of the compounds was based largely on availability and solubility in dimethy sulfoxide (DMSO) at 10 mM; DMSO is the only solvent currently used in this system. Volatile compounds were not included.

In addition to providing compounds, the NTP provided three types of assays to be used as a proof-of-principle demonstration that HTS methods could be used for acquiring reproducible concentration response data. The endpoints to be measured included: (1) cytotoxicity: the CellTiter-Glo® Luminescent Cell Viability Assay and the Cytotox-ONE™Homogeneous Membrane Integrity Assay; (2) apoptosis: the Caspase-Glo® 3/7 Assay, the Caspase-Glo® 9 Assay, and the Caspase-Glo® 8 Assay; and (3) a P-glycoprotein ATPase Assay (Pgp-Glo™ Assay) for a protein involved in drug resistance.

Dr. Tice said the first study conducted at the NCGC was to evaluate the cytotoxicity of the 1408 compounds in CellTiter-Glo® using cells of human (9 different types) or rodent (2 rat 2 mouse) origin. The cell types included those representative of different organs, and those that originated from tumors, were transformed, or were primary cells. The intent was to evaluate the response of these various cell types to a broad range of compounds.

Dr. Tice discussed structure-toxicity relationships of the compounds across the assays and representative data from different assays. He pointed out that some compounds induced toxicity in all cell types while others were uniquely active in one or a few cell types. The data are being made publicly available in PubChem, which is a free database of chemical structures of compounds and information on their biological activities. He said data analysis is just beginning with a target to identify biological and chemical fingerprints. In the future, an additional 1408 compounds will be tested in assays for key steps in pathways known to be important in immunotoxicity and cancer.

The HTS initiative will also evaluate the relationship between HTS and medium throughput screening assay data from nonmammalian species (e.g., *C. elegans*, zebrafish). NTP will establish an external advisory group for this initiative through the NTP Board of Scientific Counselors.

B. SACATM Discussion

SACATM was asked to comment on the HTS initiative in terms of its purpose, the approach being used, and/or the selection of assays, cell types, or compounds.

Dr. DeGeorge asked whether there would be any value in reducing the number of compounds to 5,000 and doing multilevel validation. Dr. Tice replied that it takes no more work to test 80,000 chemicals than 5,000 compounds because the assays are performed using robotics.

Dr. DeGeorge asked about the cost for an agency or for an academic institution to set-up the robot system and the training that would be needed. Dr. Tice said the equipment is expensive. The NCGC provides an opportunity to test a large number of compounds although so far the NTP has only provided 1400 chemicals. The Molecular Libraries Screening Network consists of the NCGC plus nine academic centers in the United States that offer different approaches for HTS. Compounds from the NTP are being made available for testing at all of these facilities through the Molecular Libraries compound repository. This collaboration will allow the NTP to identify those assays that are the most informative in terms for being part of a battery of assays for chemical prioritization and for understanding the etiology of a particular disease.

Dr. DeGeorge asked about why ethanol rather than DMSO was not used as the solvent. Dr. Tice replied that the pin tools for compound delivery used by the NCGC are currently compatible only with DMSO; however, a future aim is to test the feasibility of using other solvents. Dr. DeGeorge pointed out that DMSO suppresses cytokine production, which would an important end point in immunotoxicity testing. In terms of compound selection, he thought that good human and animal data, a chemical's prevalence and future use, and its mechanism of action should be the criteria for selection. Dr. Tice replied that the first set of 1353 compounds was selected primarily first on the basis of the availability of NTP toxicologic data, availability and solubility in DMSO at 10 mM, but the second set would be chosen more strategically.

Dr. DeGeorge thought there should be more emphasis on human cell lines. He then asked whether the chemicals are coded or the tests blinded. Dr. Tice replied that each vial has a machine code and the machine picks up the samples automatically. The technicians do not know what compounds are being tested and there is a set of 55 duplicates on each plate.

Dr. Becker considered the HTS Initiative as discovery science not hypothesis-driven science, but thought that as long as the intent is clear, the activity seemed appropriate. He pointed out that getting data on ADME--absorption, distribution, metabolism, and excretion--is a problem using *in vitro* assays. He said the challenge is not generating data, but interpreting it. He urged Dr. Tice to engage stakeholders particularly once the data are available, so that they will become comfortable with interpreting and utilizing the data within the appropriate context. Dr. Becker pointed out that there could be no validation without a prediction model. Some of the data will

need to be aggregated to test the prediction model and it must be collected in a manner that will be consistent with the prediction. Carcinogenesis is a very difficult end point, and the mode of action for different chemicals depends on species, target organs, timing, dose, and host factors; it will be a difficult biological end point to approach with *in vitro* methodologies.

Dr. McClellan was enthusiastic about the technology, but not about the NTP's approach. He thought that the current strategy did not address the ultimate objective of HTS to evaluate chemicals for their potential to cause harm or human disease, because the compounds initially identified for testing were not necessarily compounds that have been identified as causing human disease. He is concerned because discovery science has to be guided by a strategic rationale and he was not sure that there was one being applied to this initiative.

Dr. Cunningham commended Dr. Tice on the HTS activity and for looking for effects in different tissues to identify different targets. She cautioned him to be aware of the variability in measurements vs. biological variability. Measurement variability needs to limited by stringently controlling the conditions of the assays so that only biological variability can be detected. She pointed out the need for carefully controlled testing conditions and storage of the chemicals.

Dr. Dearry thanked SACATM for its comments. He agreed with many of the issues raised and said the NTP would develop a strategy for future implementation of the HTS program. He noted that there were a number of biological and technical constraints with selection of the first set of compounds, but also acknowledged that NTP's enthusiasm with wanting to forge ahead with the NCGC may have contributed to the program acting before carefully developing its strategy. The NTP is planning a workshop for the fall or winter to engage the scientific community and obtain expert input on the program. Finally, he said the NTP would assemble an advisory group to guide them in the future planning of assays, analysis of data, and selection of chemicals.

IX. Updates on ECVAM and JaCVAM

1. ECVAM

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

- Two new member states have joined the EU, which now consists of 27 countries and 500 million people.
- The EU is involved in 136 research and development projects with 260 partners.
- The public INVITTOX database contains 125 protocols for *in vitro* test methods that can be used for the European Registration, Evaluation, Authorisation and Restriction of Chemical substances (REACH) program, when no other test guidelines are available.
- ECCVAM has validated 25 test methods, has 174 methods under evaluation, and has 37 methods are undergoing evaluation for inter-laboratory reproducibility.
- The process for developing a test strategy for REACH was presented in May 2007, in a guidance document that was reviewed by 200 experts from regulatory authorities, industry, and animal welfare organizations.
- Validation of the *in vitro* micronucleus test is completed and has been accepted for REACH. A workshop on the OECD draft test guideline is planned for Washington, DC, in October of this year.

- Episkin®, an *in vitro* test method for dermal irritation, has been validated and recommended by the ECVAM ESAC as a replacement for the rabbit skin irritation test when using the EU classification system for dermal irritants. Acceptance of these assays is important for the 2009 deadline for REACH.
- ECVAM conducted a retrospective evaluation with ICCVAM on the use of four in vitro test methods for detecting ocular corrosives and severe irritants and determined that two of the test methods (BCOP and ICE) qualify for this purpose.
- ECVAM considers the LLNA as validated; it is the reference method for REACH.
- ECVAM is testing an automated assay system that uses 96-well plates to determine if it is as reproducible as manual testing. About 70 substances will be evaluated.
- The third conference on alternatives hosted by EU Commissioners Gunther Verheugen and Janek Potočnik will be held on November 5, 2007.
- Dr. Hartung proposed using an evidence-based medicine approach in toxicology. He is organizing the First International Forum Towards An Evidence-Based Toxicology in Villa Erba, Como, Italy on October 15-18, 2007. The objective is for toxicologists to discuss methodologies and problems in toxicological safety assessment, explore the available concepts of evidence-based toxicology, and launch an initiative for formal implementation of evidence-based assessment methods.

B. SACATM Discussion

Dr. Becker asked whether methods that are not fully validated are going to be implemented for REACH. Dr. Hartung responded that the REACH legislation wants to make maximum use of non-animal test methods. REACH will use validated alternative test methods to identify substances with positive and negative activities. However, REACH has concluded also that methods for which ECVAM has criteria for entering pre-validation are "suitable" to identify positive activities of substances if there is a mechanistic explanation for the response that can be used as part of a weight of evidence approach.

Dr. Becker said he was puzzled about that decision as it does not reflect using the best science and it goes against the purpose for validation. He was unsure how data from a non-validated test could be used and said the data would need to be checked using validated methods or a higher tiered test, an animal test. Dr. Hartung replied that it is a legislative decision. If a method seems "suitable," ECVAM will try to determine if the test could be validated. Scientifically he appreciates the problem, but the legislative framework is very clear.

Dr. Karen Hamernik, EPA, asked for an explanation about the accelerated validation of the micronucleus test. Dr. Hartung replied that in 2004, ECVAM used an accelerated validation approach and compiled micronucleus test data from six different validation studies plus submissions from industry. This data set was sufficient to convince ESAC to issue a validation statement. This accelerated approach took less than two years whereas classical validation studies take more than three years. Dr. Hamernik also asked how the international community would know whether a particular method had been evaluated by a full validation or an accelerated validation. Dr. Hartung said the respective classifications of the different methods are being compiled into a database.

2. Update on JACVAM

Dr. Hajime Kojima, Director of JaCVAM, presented a brief overview:

- The JaCVAM Steering Committee makes decisions on the need for validation or peer review for a particular new or revised method. 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 growth inhibition assay, Red blood cell photohemolysis assay	Peer review in progress
Skin sensitization	LLNA-DA	Validation is completed
	LLNA-BrdU ELISA	
	h-CLAT	Pre-validation in progress
Endocrine disruptor	LUMI-Cell® estrogen receptor (ER) transcriptional activation (TA) agonist/antagonist assay, CERI-HeLa-9903 ER TA antagonist assay	Planning for validation by JaCVAM, ICCVAM, and ECVAM has begun
Mutagenicity	*In vivo* and *in vitro* Comet assays for detecting genotoxic substances	Prevalidation of *in vivo* assay is ongoing Workshop for *in vitro* assay will held at the Sixth World Congress
Corrosivity	Culture model	Regulatory acceptance in progress
Skin irritation	Culture model	Planning for peer review

- The Sixth World Congress on Alternatives to Animal Experiments will be held on August 21-25, 2007, in Tokyo, Japan with Drs. Yasuo Ohno and Horst Spielmann as co-chairs. There will be 47 scientific and special sessions and up to 300 poster presentations. NICEATM and ICCVAM representatives will make a number of presentations.

X. Other Business

Dr. Freeman revisited the discussions on the NICEATM-ICCVAM five-year plan. It is clear that SACATM and others at the meeting were looking for more details in the plan including milestones and metrics to evaluate progress. NICETAM and ICCVAM need to consider this input in finalizing the plan. He suggested that the five-year plan be a recurring agenda topic for SACATM. At the next meeting ICCVAM should outline the plan and the milestones. At subsequent meetings, ICCVAM should present what has been achieved and which milestones have been met.

Dr. Dearry thanked Dr. Freeman for keeping the meeting on time, SACATM members for their insightful and stimulating discussions, and the public for their attendance. He said all the comments would be considered.

The meeting adjourned at 5 PM.