Scientific Advisory Committee on Alternative Toxicological Methods

Hyatt Regency Bethesda
Bethesda, Maryland
March 10-11, 2004

Summary Minutes

I. Attendees

The Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) met on March 10-11, 2004 at the Hyatt Regency Bethesda Hotel , One Bethesda Metro Center, Bethesda, Maryland.

SACATM Members Present

Daniel Acosta, Jr., Ph.D.
Rodger D. Curren, Ph.D.
Jack Dean, Ph.D. (chair)
Sidney Green, Jr., Ph.D.
Alan Goldberg, Ph.D.
A. Wallace Hayes, Ph.D
Nancy Monterio-Riviere, Ph.D.
Nancy Flournoy, Ph.D.
Stephen H. Safe, Ph.D.
Jacqueline H. Smith, Ph.D.
Carlos Sonnenshein, Ph.D
Martin L. Stephens, Ph.D.
Katherine A. Stitizel, D.V.M.
Peter Theran, V.M.D.
CalvinWillhite, Ph.D

ICCVAM Ex Officio Members Present

George Cushmac, Ph.D. (DOT)
Patty Decot (DOD)
Barnett Rattner, Ph.D. (DOI)
Vera Hudson (NLM)
Jody Kulpa-Eddy, Ph.D (USDA)
Joseph Merenda (EPA)
Paul Nicolaysen, Ph.D. (NIOSH)
Alan Poland, Ph.D. (NCI)
Margaret Snyder, Ph.D. (NIH)
Marilyn Wind, Ph.D. (CPSC)
Leonard Schechtman, Ph.D. (FDA)
William Stokes, Ph.D. (NIEHS)

Liaison Representative

Thomas Hartung, Ph.D. (ECVAM)

NIEHS Staff Present

John Bucher, Ph.D.
Christopher Portier, Ph.D.
Kristina Thayer, Ph.D.
Mary Wolfe, Ph.D.
Sally Fields
Debbie McCarley
Ray Tice, Ph.D.
Neepa Choski, Ph.D.
Brad Blackard
Joseph Haseman, Ph.D. (Retiree)
Christina Inhof
David Allen, Ph.D.
Judy Strickland, Ph.D.

Other Federal Agency Staff Present

Sara Shostak (NIH)
Abigal Jacobs (FDA)
Suzanne McMaster (EPA)
Dave Hattan (FDA)
Amy Rispin (EPA)
Kailash Gupta (CPS)
Karen Hamernik (EPA)
Hal Zenick (EPA)

Members of the Public Present

Troy Seidle
Sara Amundson
Sadhana Dhruvakumar
Carol Eisemann, Ph.D.

Richard Becker, Ph.D.
Mark Blazka, Dr.
George Clark, Ph.D.
Gary Cohen
Bob Sussman
Francis Kruszewski
John Gordon, Ph.D.
Martha Marrapese
Doreen Segari
Kristie Stoick
Dean Scott
Cheryl Hogue

March 10, 2004

II. Call to order and welcome

Dr. Jack Dean, chair, called the meeting to order at 8:35 a.m. on March 10, 2004, and asked the individuals in the room to introduce themselves and give their affiliation. This meeting was taped for preparation of a transcript that would be used for summary minutes.

Dr. Christopher Portier, Associate Director of National Toxicology Program (NTP), welcomed the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) and thanked them for attending the meeting.

Dr. Kristina Thayer read the conflict of interest statement for the SACATM and reminded everyone present to sign-in and register to present public comments, if applicable.

Dr. Portier provided a brief update on the status of the search for the new National Institute of Environmental Health Sciences (NIEHS)/NTP Director and recent NTP activities. He said the current director, Dr. Kenneth Olden, would retire as Director, but not as an NIEHS scientist, as soon as his replacement is chosen. Dr. Portier did not have any information on the time frame, but said the process is moving forward. With respect to NTP activities, he noted that NTP has held a number of meetings, including two NTP Board of Scientific Councilor meetings and several Technical Report evaluations and workshops. Also, NTP held two public meetings in January 2004. One public meeting was to seek input on the process of preparing the Report on Carcinogens. The objective of the second public meeting was to solicit input on the NTP Vision and roadmap for implementing the vision. The tentative unveiling of the roadmap for the NTP Vision is scheduled for November or December of 2004. Dr. Portier briefly described three programs that NTP is developing: 1) an effort to re-sequence the mouse genome for several different mouse strains to help identify key genes involved in gene/environment interactions, 2) a screen for potential developmental neurotoxicity in *Caenorhabditis elegans*, a microscopic worm and 3) a high throughput toxicity testing program. Dr. Portier finished his update by discussing the NTP Center of the Evaluation of Risk to Human Reproduction (CERHR). The CERHR has produced nine monographs and just completed its evaluation of fluoxetine (Prozac). In May, CERHR will evaluate acrylamide as a potential reproductive and developmental hazard.

Dr. Leonard Schechtman, Chair of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), welcomed and thanked SACATM on behalf of ICCVAM and the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) for their willingness to offer their time and expertise to provide advice and direction to the committee. Dr. Schechtman acknowledged the hard work and dedication of ICCVAM agency

representatives and Dr. William Stokes, Director of NICEATM, his Center staff and the contract (ILS, Inc.) staff for providing the scientific, administrative and operational support for ICCVAM's activities. Dr. Schechtman thanked Dr. Thomas Hartung, Head of the European Centre for the Validation of Alternative Methods (ECVAM), for attending SACATM and ICCVAM meetings and for promoting the continually expanding, valuable and effective ICCVAM/NICEATM-ECVAM scientific collaborations. Next, Dr. Schechtman briefly previewed some of the ICCVAM and NICEATM national and international activities that would be discussed in more detail during the SACATM meeting. These activities include several publications, such as ICCVAM/NICEATM test recommendations for agency acceptance (the Up-and-Down Procedure for Acute Oral Toxicity and *In vitro* Methods for Assessing Acute Systemic Toxicity); recommended performance standards for *in vitro* test methods (e.g., *in vitro* corrosivity test methods); ICCVAM/NICEATM test method nominations; ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods; and the ICCVAM Biennial Report. He said SACATM would also be hearing updates on ICCVAM/NICEATM test method nominations, technical evaluations of alternative test methods, and interactions with the International Life Sciences Institute/Health and Environmental Sciences Institute (ILSI HESI) Biomarker Subcommittee. In addition, SACATM would hear a presentation on the ICCVAM Strategic Plan. One effort that Dr. Schechtman said would be discussed at a future SACATM meeting is ICCVAM's entrée into the validation of "-omics"-based technologies. The first in a series of workshops assembled to address this issue was the Workshop on the Validation Principles of Toxicogenomics-Based Test Systems, held in December 2003 at ECVAM, which was co-organized, co-sponsored and co-chaired by ICCVAM, NICEATM and ECVAM. This workshop is just one example of ICCVAM's efforts to proactively expand into new scientific endeavors and increase collaborations with ECVAM.

III. Update on ICCVAM and NICEATM

Dr. Stokes, NICEATM Director, welcomed everyone and thanked SACATM for its advice. Dr. Stokes proceeded to provide an overview of ICCVAM and NICEATM activities since the August 2003 SACATM meeting.

Agency response to ICCVAM Test Method Recommendations on Acute Toxicity Testing
Dr. Stokes said the ICCVAM Authorization Act of 2000 (Public Law 106-545) requires ICCVAM to transmit ICCVAM test recommendations to the appropriate agencies through the Secretary, Health and Human Services, and requires agencies to respond to ICCVAM within 180 days. The law further requires that both ICCVAM recommendations and agency responses be made public. The first ICCVAM test recommendations forwarded to agencies in accordance with the ICCVAM Authorization Act were for two alternative methods for assessing acute systemic toxicity. These were transmitted to agencies through the Director of NIEHS on behalf of the Secretary in March 2003. All fifteen ICCVAM agencies responded, and the ICCVAM recommendations and agency responses are posted on the NICEATM/ICCVAM web site. Dr. Stokes summarized the ICCVAM test method recommendations and agency responses:

- *The revised Up-and-Down Procedure for Acute Toxicity (UDP)* – the ICCVAM recommended this method as a valid replacement for the conventional LD50[1] test for hazard classification and labeling purposes. This test method reduces the number of animals required by 60 to 70 percent compared to the conventional LD50. These recommendations were based on

[1] LD50 = Dose producing lethality in 50% of the animals (median lethal dose)

the results of an independent expert peer review panel. An implementation workshop held by ICCVAM in collaboration with the U.S. Environmental Protection Agency (EPA) and ILSI in 2002 was well attended, and included many international participants. The workshop provided detailed discussions on how to conduct both the *in vivo* and *in vitro* alternatives for acute oral toxicity.

- EPA and the Consumer Product Safety Commission (CPSC) have both announced regulatory acceptance of the recommendations. The Department of Transportation (DOT) has announced its intention to formally adopt the recommendations as well. The Organisation for Economic Cooperation and Development (OECD) accepted the method as Test Guideline 425 and it has been adopted by the United Nations Committee on Transport of Dangerous Goods. (See Discussion section following the Update on ICCVAM and NICEATM for further clarification and additional information.)

In vitro Methods for Assessing Acute Systemic Toxicity/ Guidance Document: Using In vitro Data to Estimate In vivo Starting Doses for Acute Toxicity – ICCVAM-NICEATM organized an international expert workshop in 2000 that developed recommendations for research, development and validation studies for *in vitro* screening methods (i.e., basal cytotoxicity assays), *in vitro* methods for estimating toxicokinetic parameters, and *in vitro* methods for predicting target-organ toxicity. The workshop report contains recommendations for selecting chemicals to use in validation studies for these types of methods. In addition to the workshop report, agencies were sent a copy of a related ICCVAM-NICEATM document called the "Guidance Document: on Using *In vitro* Data to Estimate *In vivo* Starting Doses for Acute Toxicity". This document provides standardized protocols for two basal cytotoxicity methods using 3T3 and NHK cells. This guidance is based on laboratory work and analyses by the German Centre for the Documentation and Validation of Alternative Methods (ZEBET), Berlin, Germany, and the Institute for *In vitro* Sciences (IIVS), Gaithersburg, MD, USA. Major contributors to the guidance document were Drs. Rodger Curren (IIVS), Manfred Liebsch (ZEBET), and Julia Fentem (Unilever Research, Shambrook Bedforshire, United Kingdom). These test methods have now been optimized for the NICEATM-ECVAM validation study, and the updated standardized protocols are available on the NICEATM-ICCVAM website. The updated protocols should be used preferentially over protocols presented in the guidance document. Based on the workshop report and the guidance document, ICCVAM made several recommendations to the agencies:

- ICCVAM recommended that cytotoxicity test data would be useful as one of the tools for estimating starting doses for the *in vivo* assessment of acute oral toxicity. One paper estimated that the use of these *in vitro* tests could achieve up to a 40% reduction in animals used per test and that fewer animals would be euthanized as a result of severe toxicity.
- ICCVAM recommended that agencies should make this information available as one of the tools that can be used to select appropriate starting doses.
- ICCVAM recommended that near-term validation studies should focus on the test methods presented in the guidance document, and that long-term activities should focus on development and validation of *in vitro* systems for estimating biokinetic parameters, metabolism, and organ-specific toxicity. These latter tests would be necessary to facilitate accurate prediction of LD50 values, symptoms of toxicity, and pathophysiological events.

In addition to the regulatory acceptance of the UDP by EPA and CPSC, other agencies concurred with the scientific validity of the UDP. Some agencies responded that Institutional Animal Care and Use Committees (IACUCs) and staff were advised of the availability of these reports and reminded that the Public Health Service policy on humane care and use of laboratory animals requires that these committees ensure that alternative methods are considered and used where appropriate. Other agencies were notified about the availability of these methods. In response to the recommendations for research, development and validation studies, NIEHS and EPA indicated that they are supporting validation studies of the two cytotoxicity methods to determine their usefulness for estimating *in vivo* LD50s and the extent that they will reduce animal use.

Dr. Stokes presented a slide showing that the use of the revised UDP plus the *in vitro* test could reduce the number of deaths per chemical by as much as 50 percent for highly toxic substances. Also, the use of *in vitro* data can reduce the average duration of the UDP test method by several days.

ICCVAM Test Method Nominations and Submissions

Dr. Stokes briefly discussed the September 2003 publication of ICCVAM's Guidelines for Nomination and Submission of New, Revised, and Alternative Test Methods. The revised document describes the process for nomination and submission of test methods, the prioritization criteria used by ICCVAM for evaluating submissions and nominations, information about performance standards, submission guidance for proposed test methods and an outline for organizing nominations and submissions. The submission and nomination guidelines are expected to facilitate the organization and completeness of test method nominations and submissions resulting in quicker review.

ICCVAM Regulatory Testing Priorities: Survey Results

Dr. Stokes reported the results of a survey of ICCVAM agency representatives with the objective of identifying testing priorities. The survey emphasized that the priorities should be consistent with mandates in the ICCVAM Authorization Act and emphasize test methods or strategies that reduce or eliminate pain and distress and reduce and/or replace animals use. Five priority areas were identified:

1) acute eye irritation/corrosion
2) acute skin toxicity (dermal irritation/corrosion, sensitization, and absorption)
3) acute systemic toxicity (oral/dermal/inhalation)
4) chronic toxicity/carcinogenicity, and
5) reproductive/developmental toxicity

This survey will guide ICCVAM when considering test method nominations and submissions.

ECVAM Collaborations

Dr. Stokes identified seven joint activities with ECVAM. The first is development of a justification for international guidance on the application of Good Laboratory Practice (GLP) to *in vitro* toxicity testing. In September 2003, the OECD Working Group on GLPs agreed to establish a working group task force for *in vitro* studies. The task force met in February 2004 to finalize an advisory document that had been developed based on information presented by ICCVAM and ECVAM to the working group in March 2003. Drs. Stokes and Schechtman from

ICCVAM and Drs. Hartung and Coecke from ECVAM participated in both the March 2003 meeting and the February 2004 task force meeting. The revised advisory document will be presented to the OECD Working Group on GLPs in May 2004 and a decision will be made to either adopt it as is or develop a more formal guidance document with an associated workshop.

The second activity involves an ECVAM-sponsored *in vitro* dermal irritation validation study. Representatives from the ICCVAM Dermal Corrosivity and Irritation Working Group (DCIWG) and NICEATM have been invited to serve as observers on the ECVAM management team for this study. NICEATM and the DCIWG will contribute to this effort by providing input on the design of the validation study and by helping to identify candidate reference chemicals for the project. NICEATM and the DCIWG are reviewing ~2400 chemicals in the EPA Toxic Substance Control Act Test Submission (TSCATS) database to identify candidate chemicals that have adequate *in vivo* data and are commercially available. Dr. Stokes said NICEATM is also conducting a retrospective review of available *in vivo* data to estimate the under-prediction rate of the rabbit skin irritation test.

The third collaboration is the joint ICCVAM-NICEATM-ECVAM Workshop on Validation Principles and Approaches for Toxicogenomics-based Methods. The workshop was held in December 2003 and co-organized and co-chaired by Dr. Schechtman and Dr. Raffaella Corvi (ECVAM). The workshop report and recommendations will be presented at the next SACATM meeting.

Two other collaborations involve strategies to replace *in vivo* acute systemic toxicity testing. In September 2003, the ECVAM Workshop on Strategies to Replace *In Vivo* Acute Systemic Toxicity Testing was held at ECVAM in Ispra, Italy. The SACATM and ICCVAM members who participated included Drs. Curren, Stitzel, Stokes, and Goldberg. The report and recommendations from this workshop will be presented at the next SACATM meeting. The joint NICEATM-ECVAM validation study on *in vitro* basal cytotoxicity methods for assessing acute toxicity is currently in its third and final phase. This validation effort involves the evaluation by three laboratories of two neutral red uptake (NRU) cytotoxicity assays: a mouse cell line (BALB/c 3T3 fibroblasts) and a primary human cell type (normal human epithelial keratinocytes) Twelve chemicals were evaluated in the first two phases, and Phase III is evaluating an additional 60 coded chemicals. The laboratory portion of this study is expected to be completed in June 2004. Dr. Stokes stated that the validation study is going well, and attributed this in part to its excellent coordination by two of the ILS, Inc. NICEATM support staff, Dr. Judy Strickland and Michael Paris.

ICCVAM, NICEATM and ECVAM will jointly participate in two future workshops: (1) Workshop on Weight of Evidence Approaches to Validation and (2) Workshop on Good Cell Culture Practices.

The final and most recent collaboration is a joint evaluation of ocular irritation assays for both *in vitro* and refinement alternatives. For this effort, ICCVAM-NICEATM will take the lead in evaluating four, current *in vitro* alternative test methods [the Bovine Corneal Opacity and Permeability (BCOP) assay, the Hen's Egg Test - Chorioallantoic Membrane (HET-CAM) assay, the Isolated Chicken Eye (ICE) Test Method, the Isolated Rabbit Eye (IRE) assay] for their ability to detect severe ocular irritants, while ECVAM will take the lead in evaluating current *in vitro* test methods for their ability to detect non-irritants and mild-to-moderate ocular irritants. In addition, both organizations will develop a shared database of high quality *in vivo* rabbit ocular

test method results. ICCVAM-NICEATM and ECVAM have designated liaisons to the respective groups of each organization (the ICCVAM Ocular Toxicity Working Group and the ECVAM Ocular Irritation Task Force).

Other ICCVAM-NICEATM Activities

Dr. Stokes briefly discussed several other ICCVAM-NICEATM activities. ICCVAM and NICEATM both have liaison members on the ILSI biomarker subcommittee chaired by Dr. Dean (Chair of SACATM). The subcommittee intends to prepare a submission for consideration by ICCVAM. Also, Dr. Stokes stated that ICCVAM-NICEATM continue to communicate with test method developers and answer questions about submissions. He said ICCVAM and NICEATM staff will present six posters and are participating in two workshops at the upcoming Society of Toxicology (SOT) meeting in March 2004.

ICCVAM Biennial Report

Dr. Stokes said the ICCVAM 2003 Biennial Report was published in February 2004. This report is required by Public Law 106-545 and describes progress made in accordance with the Act. Copies of the report are available from NICEATM, and the report can also be accessed and downloaded from the ICCVAM-NICEATM website.

Dr. Stokes concluded by acknowledging the hard work and dedication of ICCVAM agency representatives and NICEATM staff including Debbie McCarley, NIEHS, and staff from the support contractor for NICEATM, ILS, Inc.

Discussion

Dr. Green asked Dr. Stokes to clarify why the Food and Drug Administration (FDA) is not listed as an agency that indicated its acceptance of the UDP. Dr. Stokes responded that the FDA does not require LD50 testing. Dr. Schechtman verified that this is true, explaining that FDA does not specifically solicit data from LD50 or lethality tests. Further, he indicated that FDA in its response to the ICCVAM recommendation regarding UDP, acknowledged the utility of the UDP method as a substitute for the traditional LD50 test and acknowledged the potential reduction in animal usage. Dr. Schechtman then asked for additional comments from Dr. Abby Jacobs (FDA). Dr. Jacobs said FDA discourages the submission of LD50 data. If FDA does look at the data, it is not a very important component of the review process. Dr. Hayes suggested that Dr. Stokes make a note of that in his presentation. Dr. Stokes agreed and said he would do this for future presentations. Dr. Green stated that clarifying this point is important because the general public and some scientist do not know FDA's position. Dr. Green also asked Dr. Stokes why the NIEHS and EPA are the only two agencies to respond to ICCVAM research, development and validation recommendations for cytotoxicity methods. Dr. Stokes said there may be other activities, but these were the only ones identified in the agency responses and he thought it is important to acknowledge them. Dr. Stokes then asked participants if they are aware of other activities.

Dr. Stephens suggested that ICCVAM or ICCVAM member agencies could play an important role in publicly demonstrating the implementation of methods that have been accepted. Dr. Stephens was concerned that because some agencies appear to hedge their acceptance of the recommendations, they might not vigorously encourage their implementation, especially if they are perceived to be add-on studies. Dr. Stokes responded that ICCVAM is open to suggestions and reiterated some of the steps ICCVAM-NICEATM have taken to communicate the status of alternative methods, including presenting posters at SOT and holding training and

implementation workshops on the UDP and how to use *in vitro* information to determine starting doses. Dr. Alan Poland, National Cancer Institute (NCI), said that the National Institutes of Health (NIH) is also trying to communication these ICCVAM test recommendations to basic researchers. There are 12,000 external grantees in addition to the intramural program. Some of the approaches NIH is considering include communication with veterinarians at each institution and posting recommendations on web sites. Dr. Theran said he thinks this issue addresses the difficult question of how to measure the success of ICCVAM against its charter. The tools that ICCVAM develops are important, but the final measure of success is the extent to which these tools reduce and refine the use of animals. In order to evaluate success, it is critical to have some measure of how these methods are implemented in industry and how this impacts animal use. Dr. Stitzel said it is extremely important that the Public Health Service (PHS) published these recommendations. She said it is also very important that the US Department of Agriculture (USDA) inspect registered animal facilities for their consideration of these recommendations. Those two steps are very strong because the PHS and USDA regulate many animals. She also thought industry would incorporate the recommendations once the agencies state their acceptance of the new or revised methods. Dr. Stitzel was impressed at what has been done so far, and stated that getting USDA and PHS to say that a new method exists and should be used sends a very strong message. Dr. Willhite said it is appropriate that Federal regulations be promulgated to tell the regulatory community what they can and what they cannot do. Dr. Hayes asked whether there is a way to gather animal usage numbers from the agencies. For example, what are the differences in animal use for assessing acute oral toxicity before and after the UDP and *in vitro* cytotoxicity recommendations were published? Dr. Stitzel remarked that it might still be too early to measure the impact of that test method. Dr. Hayes responded that the process could be started. Dr. Dean said industry does report animal use to USDA. In response to a comment by Dr. Stitzel, Dr. Dean confirmed that the USDA animal use numbers do not include rats and mice. Dr. Stokes said those numbers for rats and mice in the United States, are not publicly available. Dr. Hayes said he thought the numbers might be available from the DOT and the United Nations (UN). Dr. George Cushmac, DOT, answered by stating that it is the shipper's responsibility to comply with the regulations and that DOT does not collect these data or receive submissions. He said that with respect to the UN, the Committee on the Transport of Dangerous Goods has model regulations that are adopted by other regulating bodies from around the world. but no data are submitted to the UN.

Dr. Acosta commented that it is very important that individuals preparing reports do a thorough literature search because often important citations are omitted and ICCVAM and SACATM should not be duplicating previous efforts. Dr. Sonnenshein agreed with Dr. Acosta, but added that sometimes what has changed is the context in which the data are interpreted. In particular, he said that in the past, the emphasis was on models that tried to reduce the complexity of human beings. The hope was that these models could inform scientists as to what was occurring at higher levels of hierarchical complexity. However, it now seems that this is probably not an accurate perception.

Dr. Stitzel reiterated how impressed she is by the ICCVAM process. In particular, she noted that years of effort were invested in trying to get the Local Lymph Node and the acute toxicity assays accepted and then in a relatively short period of time, ICCVAM evaluated and accepted them. Dr. Stizel also asked three questions. First, she asked if NICEATM is using public data from European submissions in addition to TSCATS for skin corrosion. Second, she asked why vaccines do not appear on the ICCVAM list for testing priority. Third, she asked for clarification on what is meant by a weight-of-evidence for validation. Dr. Stokes responded to the vaccine

question. The reason he believes vaccines are not on the priority list is that interest in vaccines is basically limited to the Center for Biologics Evaluation and Research (CBER) at FDA and to the USDA. He pointed out, however, that ICCVAM will give priority to this area when brought forth as formal nominations, such as the nomination for vaccine potency testing being developed by Dr. Jody Kulpa-Eddy at USDA. Dr. Hartung, Head of ECVAM, clarified the meaning of the phrase "weight-of-evidence" for validation. He said typically validation is considered to be a prospective exercise requiring the generation of new data. However, this an effort to determine the extent to which existing data can be used and this type of analysis has often been called "weight-of-evidence" validation, although ECVAM prefers the term "retrospective" validation. Dr. Hartung said ECVAM and ICCVAM-NICEATM would like to make use of both of these approaches and is organizing a workshop directed towards establishing the criteria required to make this comparison accepted as equivalent to a prospective validation effort. In response to Dr. Stitzel's first question, Dr. Raymond Tice said NICEATM is searching the TSCATS database for dermal data (and ocular data as well) as an ICCVAM activity, but ECVAM is taking the lead on searching through databases within the European community. He said the two efforts will be merged. Dr. Hartung clarified that the New Chemicals Database is an extensive, high-quality databases [3600 substances and 5600 dossiers], but it is proprietary. Although ECVAM can access the data and conduct descriptive analyses, the identity of the substances cannot be revealed without permission from the submitter.

Dr. Dean closed the session by commending ICCVAM on the progress made in the past two years (e.g., UDP and cytotoxicity) and felt that major reductions in animal use should come from full implementation of the ICCVAM test method recommendations.

IV. Update on Activities of ECVAM

Dr. Thomas Hartung, Head of ECVAM, expressed his gratitude for being invited to attend the meeting and present the recent activities of ECVAM. Because the number of collaborations between ECVAM and ICCVAM-NICEATM has expanded, Dr. Hartung said he would focus on European efforts not discussed by Dr. Stokes. He said ECVAM is in a different position from ICCVAM with respect to its mandate, politics and legislative environment. ECVAM was established following a 1986 European directive that said when an alternative *in vitro* test method provides the same scientific information as a current *in vivo* test method exist, the alternative method must be used. ECVAM assesses the validity of alternative methods and has established principles for prospective validation. In addition, ECVAM has established a database of methods that have future utility. To a lesser extent, ECVAM conducts its own research. ECVAM is in a good position to fulfill its mandate because it is neutral and independent of national or commercial interests; ECVAM has a tradition of bringing together different stakeholders. The ECVAM Scientific Advisory Committee is the only permanent advisory committee of the European Union. Dr. Hartung was pleased that representatives from ICCVAM and NICEATM are now formally considered observers to this committee.

He noted two emerging legislative policies directed towards eliminating the use of animals for safety assessments of cosmetics and other chemicals. The first is the 7th Amendment to the Cosmetics Directive that will result in the phase-out of animal experiments for cosmetics within the next 10 years, regardless of the availability of alternatives. The political expectation is that industry will provide these methodologies and ECVAM will validate the methodologies within this time frame. The second is the EU policy termed REACH (Registration, Evaluation and

Authorisation of Chemicals) that requires testing of all chemicals for basic toxicity that are produced at more than 1 ton per year (30,000 substances). The goal is to have dossiers submitted on all these chemicals in approximately 12 years. Dr. Hartung said ECVAM is restructuring itself to address these legislative mandates, specifically to increase throughput and to lower the cost of testing. ECVAM is developing a ten-year business plan that includes developing key areas of focus. ECVAM estimates that the overall program for optimizing and validating these assays, excluding development, will cost about 150 million euros over 10 years. In some areas, ECVAM has conducted its own laboratory studies, but more important is the utilization of external task forces of experts (~ 200 people). Key areas include systemic toxicity, topical toxicity, sensitization, carcinogenicity, reproductive toxicity, toxicokinetics, ecotoxicology, biologicals, the ECVAM Scientific Information Service (SIS) databases, Quantitative Structure Activity Relationships (QSARs) and strategic developments (GLP, Good Cell Culture Practices (GCCP), high throughput screening (HTS), toxicogenomics). ECVAM has increased the size of its staff over the past couple of years and is advertising for additional full-time staff in areas like biometrics, QSAR, and GLP. ECVAM is also looking for staff to develop Internet-based learning modules on available assays (E-learning).

Dr. Hartung did not want to discuss topical toxicities and skin sensitization since he considered these past successes, however he did mention that the OECD very recently issued final approval for skin corrosion and phototoxicity.

Dr. Hartung said ECVAM is also going to be taking a more proactive role in promoting the development of alterative methods by academia and industry through research activities sponsored by the Directorate General for Research and Technical Developments (DGRTD). ECVAM is taking a more proactive role in this program because both DGRTD and ECVAM have been disappointed by past output. Dr. Hartung illustrated ECVAM's proactive activities by discussing three of the most promising projects.

1. *Joint study by ECVAM and ICCVAM on Acute Systemic Toxicity*

Dr. Hartung presented data showing that the correlation between the animal LD50 and the *in vitro* IC50[2] values (expressed as log LD50 and log IC50) is relatively precise for about 70% of substances. He noted that this is a reasonable correlation, especially when one considers that the data behind this estimate are not of the highest quality (most studies were not GLP compliant, the *in vitro* data originate from different cell systems, and the estimate is based on a relatively small number of *in vitro* and *in vivo* assays). ECVAM and ICCVAM are working on a joint project to see whether the use of high quality *in vitro* and *in vivo* data can improve the correlation. Additional goals are to identify methods that are capable of predicting the starting dose for the rodent acute oral toxicity test and classifying chemicals into different hazard categories.

Dr. Hartung outlined the strategy for replacing acute toxicity testing. First, additional correlational analysis on cytotoxicity and LD50 were conducted on data available from the Registry of Cytotoxicity and the Multicentre Evaluation of *In vitro* Cytotoxicity (MEIC) study, both of which showed similar correlations. Then, ICCVAM and ECVAM agreed to jointly sponsor a validation study and held a workshop on the topic in September 2003. At the workshop, it was agreed that these test methods are a reasonable starting place, but their predictability needs to be improved, especially to capture the outliers. Strategies for improving their predictability

[2] IC50 = Inhibitory concentration estimated to affect endpoint in question by 50%

include: 1) utilization of more functional endpoints; 2) comparison of *in vitro* and *in vivo* data from the same species, such as the rat; 3) incorporation of absorption, distribution, metabolism and elimination data (ADME); and 4) evaluation of whether some types of toxicants are more difficult to predict because of their target organs (i.e., neurotoxicants). Further understanding the role these factors have on predictability may allow for correction factors or "alerts" to be added to prediction analyses.

A project proposal that represents an extension of the ICCVAM-ECVAM validation exercise and the MEIC study, called A-Cute-Tox, has been developed and involves 37 different institutions from 14 European states. A-Cute-Tox is currently under review by the European Commission and the outcome of that review is expected within six weeks. Dr. Hartung was optimistic about the review since there is no competing application. A central component of this effort is a high throughput testing (HPT) facility. The testing strategy of the HPT program is opposite of the strategy used by pharmaceutical companies, which is to test a large set of relatively undefined compounds. The A-Cute-Tox approach is to test a large number of well-defined chemicals in cell-based systems in order to optimize the correlation between the known *in vivo* data for these substances and the *in vitro* response.

2. In vitro system for evaluating chronic toxicity

Dr. Hartung presented information on ECVAM's efforts to develop an *in vitro* system to predict chronic toxicity. In 1999, a workshop on long-term toxicity was held that focused primarily on systems that would allow for longer-term exposures in cells, like flow-cell and static bioreactors. Dr. Hartung briefly mentioned an ongoing. prevalidation study of a new perfusion system called Epiflow developed in one of the framework programs by the European Commission. Dr. Hartung discussed a project called PREDICTOMICS that combines omics technology with high quality cell systems. The PREDICTOMICS project has three basic goals: 1) to develop advanced cell culture systems for the liver and kidney (i.e. co-cultures, targeted cell transformation, stem cell technology, organotypic cell cultures), 2) to identify early mechanistic markers of cell-based toxicity by genomic, proteomic and cytomic analyses, and 3) to establish and prevalidate a screening platform of toxicant-induced chronic liver and kidney disease. This project is already funded by the European Commission. He said 14 institutions from 8 European states are participating in the PREDICTOMICS project.

3. In vitro system to predict reproductive toxicity

The third project presented by Dr. Hartung is an effort to develop *in vitro* systems for the evaluation of reproductive toxicity. He said ECVAM validated three embryotoxicity tests in 2002 (a stem cell system, whole embryo culture and a micromass test) and held a workshop in 2003 on the possible regulatory use of these systems. The two main shortcomings of the ECVAM-validated tests are that 1) they only represent a partial aspect of reproductive toxicity testing, and 2) they do not incorporate metabolic systems. The validated systems are now being used as building blocks to develop other *in vitro* tests of reproductive toxicity. In 2003, ECVAM began carrying out pre-validation activity on a test method to assess testosterone production by testicular Leydig cells. Also, ECVAM is participating in an integrated project with 35 partners called ReProTect, which is an effort sponsored by the Directorate General Research. He said that ReProTect refers to "reproductive toxicology," "protection of animals" and "detection of reproductive toxicants." In current testing strategies, the reproductive cycle is covered by a variety of OECD protocols broken down by which stage of the cycle is being assessed (i.e., gamete production and release, fertilization, implantation, early prenatal development, late prenatal development, lactation and postnatal development). The structure of ReProTect is to

use existing technologies (such as *in vitro* models developed for diagnostic purposes, farm animal breeding, and by chemicals and pharmaceutical companies) and develop these into predictive tests. The various methods are organized into 4 areas: 1) pre- and post-natal development, 2) fertility, 3) implantation, and 4) technologies that cross-cut across these areas. The goal of ReProTect is to develop the existing technologies in parallel with strategic development to come up with a test battery for pre-validation and validation studies. Dr. Hartung said ECVAM is responsible for the day-to-day management of ReProTect. There is an advisory board chaired by the European Consensus Platform of Alternatives; the board has a total of nine regulatory and industry representatives. This will be the first EU-sponsored project utilizing human embryonic stem cell lines that were established in the United States more than 5 years ago. The total cost of ReProTect is estimated at 16 million euros, of which 5 percent is directed towards training and management and the rest is approximately evenly distributed among the four areas identified above.

Other ECVAM activities mentioned, but not discussed, by Dr. Hartung include: 1) an OECD draft guidance document on the application of GLPs to *in vitro* studies, 2) ongoing studies of skin and ocular irritation, 3) workshops on toxicogenomics and metabolism, and 4) efforts to establish a cell transformation assay(s) to detect non-genotoxic agents.

Discussion

Dr. Green asked Dr. Hartung to clarify whether the chronic toxicity efforts distinguished between chronic and subchronic toxicity. Dr. Hartung responded that it is too early to distinguish between the two because ECVAM is in the earliest stages of developing strategies and at this moment they are exploring technologies that might play a role in the final strategy. A workshop is being planned for this fall to develop strategies for chronic toxicity testing where he expects these strategies to be discussed. Dr. Dean asked what strategy will address absorption, metabolism and distribution. Dr. Hartung responded that ECVAM had a workshop on this issue very recently with 28 experts. The experts had three main suggestions. First, assays and predictive systems should be developed that can give an indication of whether a compound is metabolized. Second, the experts discussed the utility of transfected cell lines. Third, the experts thought that QSAR and physiologically based pharmacokinetic (PBPK) models should be used. The workshop report should be available shortly. Dr. Dean asked for further clarification on whether a metabolite identified from QSAR would be the compound used in an assay. Dr. Hartung responded that this would not necessarily be the case for chemical or cosmetic ingredients (in contrast with pharmaceuticals) because it would be difficult to synthesize and then test a metabolite. Also, Dr. Hartung noted that characterizing the metabolites may not necessarily be hazard identification. Dr. Dean thought it would be critical to establish whether a negative *in vitro* finding is due to inadequate characterization of the metabolites. Dr. Hartung agreed and said metabolism may not play a major role in acute systemic toxicity, which may reflect an “overloaded” system. He also noted that the extent to which metabolism is important in chronic toxicity is not fully established and testing a sufficient number of chemicals in systems competent with respect to metabolism will guide future use of biopredictors like QSAR.

Dr. Goldberg complemented Dr. Hartung for his work at ECVAM and asked whether ECVAM is trying to gather information about proprietary methodologies that have been internally validated within the company where they are being used. Dr. Hartung thanked Dr. Goldberg for his comments and responded that the most sophisticated *in vitro* systems are being used for decision making by regulators and not by the pharmaceutical industry. He said there is an effort

in Europe to form an industry foundation (agrochemical, chemical, and pharmaceutical industries) since they have similar pressure for testing. In addition, the EU is co-sponsoring an effort to establish a scheme for sharing data with industry to allow for access to proprietary data, such as *in vivo* data and their respective reference chemicals.

Dr. Willhite raised several points. First, he asked for additional information on the three validated embryo toxicity tests. Next, he asked Dr. Hartung about two recent papers that concluded that QSAR methods like TOPCAT and CASE/Multicase are not reliable tools to predict reproductive and developmental toxicity. Dr. Hartung responded that there are approximately 3000 QSAR models and none has been through a formal validation process and the time is right to apply the ECVAM validation criteria to these models. Efforts to validate QSAR models are now being carried out under the umbrella of OECD; there was a meeting last March to try and develop validation criteria that apply to QSAR. This is especially true in Europe where they would be proposed as a replacement to *in vivo* and *in vitro* tests. Dr. Hartung is hopeful that the combination of increased access to *in vivo* data and the use of models based on data from high quality, validated *in vitro* assays will aid the development of QSARs.

Dr. Curren complemented Dr. Hartung on the efforts of EVCAM, noting that ECVAM has the most aggressive and directed efforts on *in vitro* activities. Dr. Curren asked whether interim information about ECVAM projects would be available, so that others around the world can use this information as they develop their own approaches to alternatives. He said such an approach could also benefit the European efforts. Dr. Hartung agreed.

Dr. Sonnenschein also congratulated Dr. Hartung and asked for clarification about a presentation slide [in presentation, but not discussed] not discussed that referred to a cell transformation assay to be used for predicting carcinogenicity. Dr. Sonnenshein felt these types of assays are unreliable and generally not useful. Dr. Hartung responded that an OCED draft guideline is in preparation on this topic and ECVAM feels that it is important that these tests undergo formal pre-validation/validation to establish their potential utility before a guideline is published.

Dr. Stephens congratulated Dr. Hartung on his work and said he is impressed with ECVAM. He noted that ECVAM is looking at technologies that can be developed in the long-term to address current technological barriers. Dr. Stephens suggested that ICCVAM should adopt a more proactive strategy. Dr. Dean concluded the discussion by saying that it is encouraging to see the progress made by ECVAM and also the progress resulting from the collaboration between ICCVAM and ECVAM.

V. Toxicology in the 21st Century: The Role of the National Toxicology Program

Dr. Portier presented the NTP Vision for the 21st Century ("the Vision") and the timeline for developing the roadmap on how to implement the Vision. Dr. Portier said the NTP is the world's largest toxicology program and three Federal agencies participate in the NTP: the Centers for Disease Control (CDC), the Food and Drug Administration (FDA), and the National Institutes of Health (NIH). NIEHS is the lead agency and NIEHS and NTP share the same director. The NTP spends approximately $140 to $165 million a year on toxicology testing, research testing, and other activities (not all of which involves animal testing). Dr. Portier said the NTP is starting

its 26th year and would spend 12 months, beginning October 2003, formulating its future role in toxicology. He noted that in contrast to data generated by pharmaceutical companies and agribusiness, NTP data are in publicly available databases.

Dr. Portier said the NTP Vision has already been determined and NTP is now in the process of creating a roadmap to move the NTP closer to the Vision. The NTP Vision is "to move toxicology from a predominantly observational science at the level of disease-specific models to a predominantly predictive science focused upon a broad inclusion of target-specific, mechanism-based, biological observations." At this time, the NTP is seeking comments on how to implement the Vision. NTP has already solicited public comments via a Federal Register notice and public meeting. Also, the NTP is receiving scientific input from three separate committees: 1) an internal NIEHS Working Group; 2) an NTP Executive Committee Working Group with representation from eight Federal agencies; and 3) a Board of Scientific Working Group. Dr. Portier said the NTP is also seeking advice from outside experts to speak to these groups and will organize a retreat to discuss the advice received. The roadmap should be released to the public in fall of 2004 or early winter 2005.

Dr. Portier discussed seven basic activities that could be considered for the NTP vision.

1. *Rapidly develop better models, faster screens*
2. *Move from disease-specific focus to systems/mechanism-based focus*. This will require NTP to consider several factors to a greater extent than they have been considered in the past (e.g., exposure timing, genetic controls on response, system wide evaluation of data and the development of tools for integrating scientific data).
3. *Develop better and broader baseline information*. The biggest problem in the development of alternative methods is the lack of data. NTP can help address this by developing high throughput methods to test a greater number of compounds. These methods can also be used to assess chemicals that have already been tested (cancer bioassays, developmental toxicity, genotoxicity).
4. *Enhance development of multidisciplinary/multi-agency scientific teams*.
5. *Cross-link disease focus with mechanism focus*.
6. *Expand the linkage between toxicology and basic science to enhance both areas*.
7. *Develop training programs to meet the needs of a broader-based NTP*.

Dr. Portier briefly mentioned similar efforts by other agencies, including a National Academy of Sciences committee sponsored by NIEHS charged with advising NIEHS and a Federal regulatory agency liaison group on issues related to toxicogenomics and other omics. He noted that EPA is also looking towards toxicology in the future, including how to deal with genomics, high throughput screening and QSAR.

Public Comment

Dr. George Clark of Xenobiotic Detection Systems thanked Drs. Portier and Hartung for their presentations and said NTP and ECVAM are on the right track. Mr. Troy Seidel, People for the Ethical Treatment of Animals (PETA), also commended ECVAM and made two points. First, he said that it would be beneficial to prepare a list of sponsors for some of the collaborative efforts between ECVAM and U.S. agencies. Second, he said it is difficult to understand how efforts by NTP and ICCVAM will come together.

Discussion

Dr. Smith was very impressed with the efforts directed towards alternative methods in both Europe and the United States. She asked for clarification on how the methods presented would be used given that they focus on hazard identification and do not address exposure and dose response. Dr. Portier presented two examples of where NTP is considering dose-response relationships for alternative methods. The first is a *C. elegans* screen for developmental neurotoxicity that will look at responses over a wide dose range in addition to length of exposure. The second is the incorporation of *in vitro* assays that address absorption, distribution, metabolism and elimination (ADME) issues into a high throughput system.

Dr. Acosta asked what the Federal agencies, especially NIH, are doing to communicate with the public, including schools K through 12. He also wanted to know whether the NTP vision includes a strategy to promote better understanding of toxicology to the public. Dr. Portier responded that toxicology is included in a NIEHS grants program that focuses on environmental health sciences and K through 12 education, but this is not something that NTP has addressed historically. Dr. Portier said he would consider this advice.

Dr. Hayes asked for clarification on what Dr. Portier meant by "cross-link disease focus with mechanism focus." Dr. Portier responded that there may be common mechanistic targets that are important for a variety of toxicological endpoints. For example, an effect on cell differentiation may be important for cancer in one organ and a developmental defect in another. Dr. Hayes questioned how such a large number of endpoints could be incorporated into a rapid screening process. Dr. Portier replied that this is not clear and is why the NTP is seeking advice. Dr. Portier added that this is why high throughput methods are a priority. He said even though this entire process may not lead to better tools for decision making, it's a question that needs to be answered.

Dr. Sonnenschein commended Dr. Portier and his staff for addressing the issue of what NTP should be doing in the 21st century, but wondered how NTP would evaluate existing information that indicates the situation is very complex. By way of example, he said that the human genome project has shown that the one gene-one protein model is not correct. Dr. Portier responded by saying that he thinks toxicology is at the point where it is appropriate to reflect on the science and make some decisions about what to do in the future. Further, the NTP is going to continue much of what it does currently until there is some degree of certainty about replacing existing tests. Dr. Theran commented that he appreciated the complexity of what NTP is trying to do, but thought that is important to have a way of measuring impact on the three Rs (the reduction, refinement and replacement of animal testing). He said it is important to find a way to get animal use numbers to measure effectiveness and communicate the impact to stakeholders. Dr. Portier replied that it will be very difficult to come up with a measure of impact. Even if the numbers were available, it might be difficult to measure impact in the short-term since the number of animals used to test a given compound might be reduced, but offset by a greater number of chemicals tested. Dr. Stitzel was very supportive of the NTP Vision, but emphasized the importance of having the support of the public, government agencies and industry. Dr. Goldberg commented that he thought the single, most important question Dr. Portier asked is how we begin to make sense of the available scientific data and use it to develop a product that will implement the three Rs.

Dr. Safe felt the agencies are doing a poor job of communicating information on hazard and risk to the public and that should be a significant portion of the vision for toxicology in the 21st

century. Dr. Portier responded that NTP conducts hazard assessments and risk communication is really the purview of Federal agencies in their legal mandates. Dr. Stephens said he is excited about the NTP vision, especially the references to improving or replacing current tests with faster, mechanistic-based assays. However, he was concerned that the statements in the presentation about the need to maintain scientific quality and clarity during the transition give too much credibility to current methods that have been questioned for their scientific quality, such at the two-year bioassay. Dr. Hartung said he is impressed with the program and noted the opportunities for collaboration between ECVAM and NTP given the similarities between the field of alternatives to animal experiments and the NTP Vision. Dr. Dean asked for clarification on how the NTP plans to translate its research into practical applications. Dr. Portier responded that NIEHS may have grants that help create translational tools, but also the agencies will have to aggressively promote translation because they have to be responsive to their own laws. He also said he suspected that industry groups would invest their own time and effort into developing tools for translation of information given the potential use of these methods in making public health decisions. Dr. Stitzel asked if NTP is going to make an attempt to understand basic mechanisms, such as linking all data known on receptors. Dr. Portier said that NIEHS/NTP has been spending a significant amount of money on this type of research, which he called high output assays (genomic, proteomic and metabonomic assays that measure thousands of endpoints form single samples). Dr. Willhite was disappointed to hear that the total usage of animals may not decrease because reductions in animal use for each compound may be offset by testing a greater number of chemicals. He emphasized the importance to the SACATM of being able to measure success in terms of reducing and replacing animals.

Dr. Sonnenschein asked whether the use of screening technologies by the pharmaceutical industry had really led to significant breakthroughs in drug development. Dr. Dean responded that screening does lead to breakthroughs in drug development, but explained that the use of screening in toxicology differs from screening in the pharmaceutical industry. In the pharmaceutical industry, the screen would be against a known target, whereas a toxicology screen is much broader. Dr. Curren made two points. First, he thinks a more appropriate measure of progress would be if new methods improved the prediction of human toxicity rather than just reduced or refined animal usage. Second, he asked if there would be other opportunities for SACATM, the public, and others to comment on the roadmap as it becomes more developed. Dr. Portier responded that there would be three other opportunities for comment: 1) the NTP Board of Scientific Counselors meeting in June, 2) a public meeting when the roadmap is released, and 3) once more before the roadmap is implemented. If SACATM meets prior to the release later this year, then there would be another opportunity for SACATM to comment. Dr. Goldberg made two comments. First, he was concerned that issues of pain and distress are not getting enough attention. Second, he said that SACATM needs to recognize that not all alternative methods used by industry and in academia have regulatory applicability and that the focus should be on methods used in the regulatory arena.

VI. ICCVAM Strategic Planning Process

Dr. Schechtman presented the ICCVAM Strategic Plan: Mission, Vision, and Strategic Priorities. The draft Strategic Plan, the ICCVAM mission, and the ICCVAM vision statement were developed at a meeting in January 2004, attended by 30 individuals representing 14 of the 15 ICCVAM member agencies. The ICCVAM mission and vision statements were unanimously approved by all of the ICCVAM participating agencies on February 11, 2004. These statements

are based on discussions of ICCVAM's strengths, challenges and areas for improvement, and organizational issues, bearing in mind the directives of the ICCVAM Authorization Act of 2000. Numerous ICCVAM strengths were identified, some of which include the strong foundation and clarity of mission provided in the ICCVAM Authorization Act; the leadership and capability of ICCVAM and NICEATM; ICCVAM's role as a central resource for validation-related activities; the expertise and commitment by ICCVAM representatives; its interactions with animal advocacy groups; and its strong and effective international ties, especially with ECVAM. Dr. Schechtman discussed several key challenges that ICCVAM faces, such as the need to be more proactive in stimulating the development of new test methods and prioritizing their review; achieving the proper balance between being responsive to the three R's and to human health objectives and environmental issues; functioning effectively with limited resources dedicated to NICEATM and agency-specific obligations and time constraints faced by ICCVAM agency representatives; further optimizing ICCVAM's internal processes in order to further its operating efficiency; maintaining continuity and effectiveness of process as ICCVAM representatives are replaced with new people; continuing to communicate effectively with stakeholders so as to ensure transparency, visibility, credibility, public and industry awareness, cooperation and support; clarifying the relationship between ICCVAM, SACATM, NICEATM and NTP; and strengthening international relations/partnerships with Europe and the Pacific Rim.

ICCVAM's Mission and Vision

Dr. Schechtman then presented the ICCVAM Mission:

> ICCVAM's mission is to facilitate development, validation, and regulatory acceptance of new and revised regulatory test methods that reduce, refine, and replace the use of animals in testing while maintaining and promoting scientific quality and the protection of human health, animal health, and the environment.

Dr. Schechtman presented the three components of the ICCVAM Vision. First, ICCVAM will be recognized as a leading authority on test method development and validation both within the Federal government and internationally. Second, ICCVAM will play a leading role in six activities: 1) promoting high quality science as the basis of national and international regulatory policy; 2) setting and harmonizing international standards for scientific validation of test methods; 3) promoting and facilitating development of priority alternative test methods; 4) identifying key alternative test methods and strategies and facilitating their validation and acceptance; 5) fostering human and ethical approaches to testing that replace, reduce, and refine the use of animals; and 6) promoting awareness and adoption of scientifically validated test methods by regulatory agencies both nationally and internationally. The third component of the ICCVAM vision is that ICCVAM will develop the internal and collaborative capacity to 1) ensure the scientific quality and integrity of its work; 2) implement reliable processes and operating procedures that are credible, effective and efficient; 3) build national and international partnerships with governmental and non-governmental groups, including academia, industry, advocacy groups, and other key stakeholders; and 4) secure the necessary human and financial resources to effectively carry out its mission.

ICCVAM's Strategic Map for 2004 - 2006

Dr. Schechtman presented the components of the ICCVAM draft Strategic Map (i.e. Central Challenge, Strategic Priorities, and Strategic Objectives) and the process ICCVAM used to create the map. The Strategic Map, which is to serve as a "roadmap" for ICCVAM to follow for the next three years to facilitate ICCVAM fulfilling its Central Challenge, was adopted as a draft

by ICCVAM in February 2004 and is considered to be a "living" document meant to be revisited periodically and revised as necessary. Dr. Schechtman stated that ICCVAM's Central Challenge is "to strengthen ICCVAM's impact nationally and internationally." This Central Challenge is underpinned by six Strategic Priorities, i.e. 1) set priorities for evaluating test methods and carry out reviews, 2) facilitate collaborative scientific validation internationally, 3) stimulate development of priority test methods and strategies, 4) foster appropriate use of validated test methods, 5) strengthen ICCVAM's capability and sustainability, and 6) strengthen interaction with stakeholders. This last Strategic Priority is the most cross-cutting and is an essential factor in the implementation of all of the other ICCVAM Strategic Priorities. Strengthening its interaction with its stakeholders is viewed as a means of further improving ICCVAM's effectiveness and efficiency, broadening its international collaborations, stimulating test method development and test strategies, promoting an awareness of validated methods, and building a strong capability base and securing sustainable resource support. Each Strategic Priority is supported by a rationale and Strategic Objectives, and the accountabilities for implementation of each were identified.

Dr. Schechtman said having adopted its Mission, Vision, and Strategic Priorities in February 2004, ICCVAM is now prepared to initiate the implementation process. ICCVAM's approach would involve adoption of the final Strategic Map at its next meeting, establishment of member Working Groups that will develop a course of action by which to implement the Strategic Plan, and the review, discussion, and revision (as needed) of the Implementation Plan. Presently, ICCVAM is seeking input from SACATM and the public for consideration in developing the final version of the ICCVAM Strategic Plan, i.e. Mission, Vision and Strategic Priorities, including the Strategic Map. It is expected that the finalized Strategic Plan and key aspects of the Implementation Plan will be available for the next SACATM meeting.

Public Comment

Dr. George Clark, Xenobiotic Detection Systems, discussed issues related to the funding and validation of test methods from the perspective of small business. He made five main points. First, he said the development of test methods has little market potential for conventional forms of funding. Second, government is the primary source to fund areas of research for public health. The NIEHS funds Phase I (proof of concept) and Phase II (method development) Small Business Initiated Research (SBIR) as a mechanism for small business entities to propose test methods. Third, there is no Phase III SBIR (validation) process in place that would allow the validation of such methods and facilitate their adoption for regulatory purposes. Fourth, Dr. Clark suggested that SACATM act as a peer review body to bring methods to ICCVAM and NICEATM for validation. Finally, he thought there should be representation of small business during such discussions.

Discussion

Dr. Hayes expanded on two points raised by Dr. Clark's presentation. First, laws are driving the development of alternative test methods in Europe in contrast to the United States. Second, these tests are very, very expensive to validate. He thought ICCVAM's strategic plan should address issues of small business groups.

Dr. Curren summarized pre-presentation thoughts of the lead discussants for this agenda topic (Drs. Curren, Stitzel, and Stephens). Overall, the lead discussants were pleased to see the ICCVAM take the initiative to develop a strategic plan. The lead discussants had some questions of a clarifying nature. First, they wanted to know how input from outside stakeholders

entered into the plan. Dr. Curren said Dr. Schechtman's presentation clarified this issue, but given that the plan was presented as an adopted plan and not a draft plan, it is unclear how comments from SACATM might be incorporated. Dr. Schechtman responded that because the strategic plan relied heavily on the ICCVAM Authorization Act, which was really the culmination of efforts by non-agency stakeholders, ICCVAM felt they had public input from the beginning. He also clarified that the Strategic Plan is not a fait accompli. Dr. Stokes elaborated on this point saying that that ICCVAM just identified its strategic priorities and supporting strategic objectives; however, ICCVAM has yet to develop the strategic plan that will state what, how and when they want to accomplish these objectives, and is seeking public input on the development of the strategic plan. Dr. Portier asked for clarification on whether the strategic plan addresses the issues of the multiple agencies involved in ICCVAM, or whether it addresses the issues of ICCVAM as an interagency committee. Dr. Curren said he thought there should be some interaction between the SACATM Strategic Planning Working Group and the ICCVAM strategic planning program. Dr. Curren presented other comments from the lead discussants. One point is that the ICCVAM Mission Statement seemed to emphasize alternative methods (those that result in the reduction, replacement and refinement of animal use) and not the "new" and "revised" methods cited in the law. Dr. Stokes said ICCVAM considered the mission statement to be consistent with the law. Dr. Curren responded that ICCVAM may need to think about the wording in the mission because the law speaks of new, revised, or alternative methods.

Dr. Stephens summarized four comments from the lead discussants on the ICCVAM Vision. First, the discussants thought that the first bullet - that ICCVAM would be recognized as a leading authority on test method development and validation both within the Federal government and internationally – may not be the appropriate starting place. He said a strategic process for setting priorities about which methods should move through the process should precede the issue of test method development and validation. The lead discussants suggested that ICCVAM add a bullet early in the vision saying something to the effect of "gather the necessary background information and devise criteria for setting priorities for assay development and validation." Second, they thought the vision should put more emphasis on regulatory acceptance and utilization of alternative methods. They proposed a bullet early in the vision along the following lines: "facilitate the regulatory acceptance and adoption of validated test methods by ICCVAM member agencies." Third, with respect to the first bullet in the vision, they wondered whether ICCVAM needs to be an authority on test method development, or should it be the conductor that oversees the process at each step in the validation chain. Finally, they had a comment on the third component of the vision regarding the statement "secure the necessary human and financial resources to effectively carry out its mission." The lead discussants thought that a statement about ICCVAM developing a plan and rationale for its activities over a three to five year period would lead to strategies to secure the resources for those activities.

Dr. Stitzel presented comments from the lead discussants on ICCVAM's Central Challenge ("to strengthen ICCVAM's impact nationally and internationally") and priorities. She said the lead discussants thought one of the key central challenges is for ICCVAM to understand what is needed and where the most efficient use of resources (financial and human) would be to reduce animal use and develop better tests. The lead discussants had four comments on ICCVAM priorities. They thought the first priority should be to understand the current situation. Another priority should be to stimulate the development of new methods. A third priority should be to strengthen interaction with stakeholders and assist agencies to assure that submissions contain data from new and approved methods. Another priority should be for ICCVAM to understand

barriers to the process; for example, how to obtain good historical data to compare the quality of current and new test methods.

Dr. Hayes questioned whether public perception should be considered an ICCVAM strength, since ICCVAM is not known to many in the public. Dr. Hayes made three additional comments. First, he asked if ICCVAM is going to elaborate on how it plans to secure the necessary human and financial resources. Second, he wanted to better understand the relationship between ICCVAM, NTP and NIEHS. Finally, he asked for clarification on who SACATM advises. In response to the question about resources, Dr. Stokes said the parent organization for ICCVAM is NICEATM and that NICEATM has a budget. Most of NICEATM's work is conducted by contract staff funded by NIEHS, but there is a mechanism for NICEATM to accept support from other agencies. This would be the mechanism for the committee to get support to carry out its activities. The law specifies certain ICCVAM activities, but many of these activities require support through NICEATM. Dr. Portier added that the NIEHS is the primary agency under the ICCVAM Act of 2000 and developed an implementation plan that specifies the responsibilities of each party under that Act. Dr. Portier was unsure whether the implementation plan is public, but would check and see if it could be distributed. Dr. Portier clarified that SACATM provides advice to the directors of 15 Federal agencies through their representatives on ICCVAM. NIEHS is both a member of ICCVAM and manager of the process. Other agencies have their own roles as members of ICCVAM. Dr. Dean read an excerpt from the law that said SACATM advises ICCVAM and NICEATM. Dr. Portier said that since NICEATM is under the director of NIEHS and NTP, SACATM effectively provides advice to the director of NIEHS and the director decides what resources go to NICEATM. Dr. Portier further clarified that SACATM should be providing advice to NICEATM/NIEHS on what they should be doing to manage ICCVAM scientifically and to the individual agencies or their designees on what ICCVAM should be doing scientifically. Dr. Smith strongly endorsed the central challenge to facilitate national and international adoption of new, revised or alternative test methods, especially since companies have to meet international standards. Dr. Stitzel said the lead discussants for this topic didn't disagree with this point, but they weren't sure that was the most important challenge and that it may be inappropriate to focus on international acceptance. Dr. Merenda, EPA, said possibly the international focus developed in response to the facilitator of the ICCVAM Strategic Planning meeting charging the participants to look ahead at the next five years. When ICCVAM representatives did that, they recognized that it would be critical for ICCVAM to collaborate extensively with ECVAM and it would be a disservice to focus exclusively on the needs of the United States. Dr. Schechtman added that ICCVAM felt that making the central challenge too focused on the immediate and not more broadly applicable would restrict ICCVAM's ability to build the necessary priorities and objectives under each Strategic Priority. Dr. Curren said the lead discussants interpreted a central challenge as being something that is the most difficult to accomplish and he was not convinced the current central challenge is the most difficult. Dr. Schechtman asked if it would be helpful for ICCVAM to better define what is meant by "central challenge," "strategic priority," etc. Drs. Curren and Stitzel said the wording of the current central challenge sounded like the United States is trying to compete with Europe rather than help address the international challenge.

Dr. Goldberg asked if ICCVAM considered the NTP vision was considered by ICCVAM and how to implement the Vision as it relates to ICCVAM. Dr. Stokes said that the NTP Vision was distributed to ICCVAM and that ICCVAM recognizes that the new test methods developed as part of the vision, at least those with regulatory applicability, will have to be evaluated for their scientific validity and this will be a challenge. Dr. Green asked if it were true that the strategic

priorities were presented in no particular order and ICCVAM is seeking input from SACATM on determining the order of priority. Dr. Schechtman confirmed this. Dr. Wind, CPSC, added that the objectives under each priority were also not prioritized. She said the only reason that strengthening interaction with stakeholders was the last priority was because it touched on all the other priorities. Dr. Wind also pointed out that "national" preceded "international" in the central challenge and that the central challenge was discussed for a long time at the strategic planning meeting. Dr. Stokes said the central challenge should not be considered in isolation, but in the context of the mission and vision statements. Dr. Stitzel said she wanted to make it clear that the lead discussants are very pleased that ICCVAM developed a strategic plan and that it is a great effort. Dr. Portier emphasized that by law ICCVAM is the lead authority on validation in the United States, so the only question is the role of ICCVAM internationally. Dr. Dean thanked the ICCVAM representatives for developing a strategic framework and acknowledged the extensive discussion on this topic by SACATM.

VII. Update on Animal Use: USDA Research Facility Reporting Requirements

Dr. Kulpa-Eddy, USDA, summarized the Research Facility Annual Reports on Animal Usage submitted to the USDA. She said this is one type of tool that can be used to measure success of the alternatives program. In 1966, Congess passed the Laboratory Animal Welfare Act that did not require annual reporting. In 1973, following amendment of the Animal Welfare Act, the USDA began publishing reports of animal usage. Dr. Kulpa-Eddy said registered research facilities (e.g., colleges, universities, pharmaceutical firms, contract laboratories, etc.) and government agencies that use animals (e.g., Department of Defense and the US Fish and Wildlife Service) are required to report. However, the definition of "live animals" used by the USDA does not include every type of invertebrate or vertebrate. Instead, the definition is limited to warm-blooded species, so fish, amphibians, insects and reptiles are excluded. Also, not every warm-blooded species is included; laboratory mice, laboratory rats, and birds are excluded. The language of the amended Laboratory Animal Welfare Act asks for the number of experiments conducted involving necessary pain and distress. Therefore, early reports did not include number of animals, but rather the number of experiments. Dr. Kulpa-Eddy said reports now present number of animals used; however these numbers do not represent the total numbers of animals used (referring to the above statement regarding excluded species). She noted that early reports to Congress indicated that almost all incidences of unrelieved pain involved the mandated type(s) of testing that ICCVAM considers priority (i.e., research and development/quality control/safety testing of medicines, cosmetic products, and chemical products). Two other reporting categories have been added since the late1970s: 1) Category C, where no pain or distress is involved and 2) Category D, a refinement of animal use where pain or distress is alleviated by drugs. Overall, the number of animals reported has gone down, although the number in Category E (experience unrelieved pain and distress) has been fairly constant at approximately 6 to 8 percent of the total number of reported and regulated animals. Dr. Kulpa-Eddy said the large increase in the use of rats and mice over time is not reflected in these numbers. The USDA also requests an explanation for why drugs cannot be used to alleviate pain and distress in certain procedures. Of the animals in Category E, almost two thirds of the animals are reported due to mandated testing as opposed to basic research procedures. Dr. Kulpa-Eddy identified two reasons why animals are falling into this category for basic research. One is that the vast majority of animals in this area are used for drug and vaccine development, tests that regulatory agencies will likely never see. Also, the USDA has asked that people who use complete Freund's adjuvant to report these animals as Category E.

Dr. Kulpa-Eddy said when you look among government agencies, the USDA accounts for the largest number of animals in Category E, because of vaccine testing mandated by the Center for Veterinary Biologics. She also said there is likely to be some overlap in animal use among agencies; for example, some contract labs report the animals used for skin sensitization under both EPA and FDA testing, thereby resulting in an over-reporting of animal use.

Dr. Kulpa-Eddy then presented information about the number of specific species used in mandated testing and how they are used, such as dogs (investigational/new drug application and rabies vaccine testing), cats (feline upper respiratory and rabies vaccines, investigational/new drug applications and acute oral toxicity testing), guinea pigs (diphtheria/tetanus human vaccines, skin sensitization, clostridial bacterins for animals), hamsters (Leptospiral bacterins), rabbits (primary eye irritation, acute skin irritation and/or corrosion, developmental toxicity), non-human primates (drug toxicity/safety/kinetic studies), farm animals (vaccine and drug development) and other mammals (mink—vaccine testing; wild rodents—rodenticide efficacy). She said approximately 40,000 hamsters are used annually, essentially all for "Leptospiral bacterins" vaccine potency testing. Dr. Kulpa-Eddy concluded by saying that some facilities report the number of rats and mice even though they are not required. Based on these reports, rats and mice may be accounting for 90 to 95 percent of animals used in research currently.

Discussion

Dr. Dean began the discussion by commenting on the relatively high numbers of animals used in the diphtheria/tetanus human vaccine mandated testing program and asked about the type of test (e.g., release or efficacy test). Dr. Kulpa-Eddy did not know, but suggested that someone from the Center for Biologics at the FDA might know. Dr. Hayes asked who used cats in acute toxicity testing. Dr. Kulpa-Eddy responded that she assumed some researchers chose to use cats when fulfilling the rodent and non-rodent species acute toxicity testing requirement. Dr. Willhite asked why rats and mice are not reported. Dr. Kulpa-Eddy replied that the USDA does not have the authority to ask for that information. An amendment to the Animal Welfare Act in the 2002 Farm Bill adopted by Congress specifically excluded these species. Dr. Stephens thanked Dr. Kulpa-Eddy for her analysis. He said he'd conducted similar analyses and confirmed many of the trends Dr. Kulpa-Eddy reported. Dr. Stephens made two additional points. First, that as a committee, SACATM may need to recommend changes to the U.S. reporting system or use overseas numbers to get a clearer idea of animal usage because these data are very valuable. Second, he said it is important to recognize that animals not in Category E could also experience pain and distress at some point in an experiment. Dr. Theran said he thought the research and testing facilities likely have information about the numbers of rats and mice used, but this is an access problem since they are not required to report this information. He said a large number of animals aren't being reported and SACATM really needs this information. Dr. Poland made the comment that many mice used in research are for breeding transgenics, so knowing the total number of animals used might not say much about experimental uses. Dr. Goldberg made two comments. First, it's clear from construction on academic campuses that the numbers of mice, mainly transgenics, has increased rapidly in recent years. Use of other species seems to be more stable. His second point was that he thought ICCVAM or SACATM should explore strategies to obtain voluntary disclosure of the numbers of rats and mice used. Even though these numbers may not be easily interpreted, it would be a better estimate than the current one. Dr. Dean asked if facilities are prohibited by law from disclosing these numbers. Dr. Goldberg responded that there is nothing mandated that says an institution can't release these numbers. Dr. Snyder said the Office of Management

and Budget would have oversight over any new questionnaire and would evaluate the questionnaire for factors such as regulatory burden and educational time required to complete the form. Dr. Snyder added that ICCVAM's purview is regulatory testing. The overall numbers from such a questionnaire would reflect all research and likely what should that concern ICCVAM and SACATM. Dr. Snyder also said that reducing the total number of animals used in research is not necessarily the goal. Instead, the commitment is to reduce the numbers within each test to the minimum required to provide scientific validity.

Dr. Nicolayasen made four comments. First, animal use is voluntarily reported to the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). Second, it is difficult to categorize whether an animal is experiencing distress. Third, only drugs are considered as a way to alleviate pain and distress, so other techniques would not count. Fourth, Dr. Nicolayasen raised the minor point that it is impossible to know how accurate the column in the USDA report is for presenting the number of animals being bred, conditioned, or held for use, but not yet used. Dr. Dean closed the discussion by thanking Dr. Kulpa-Eddy for a nice presentation.

VIII. Update on ICCVAM Recommended Performance Standards for *In vitro* Dermal Corrosivity Methods

Dr. Rispin began her presentation saying that what ICCVAM has accomplished with performance standards for *in vitro* methods will lay the foundation for getting companies to come into the marketplace with "me-too" methods. However, endorsing a proprietary method (Proprietary Test Methods or PTM) that was developed for marketing for profit is a challenge to Federal agencies. A committee was formed under ICCVAM to develop a strategy for dealing with PTMs. Lawyers advised the EPA that if a PTM is of interest to EPA for regulatory purposes, then EPA must write a generic test guideline that spells out performance standards that must be met. The performance criteria pertain to the specific validated proprietary method and also to any other future proprietary test method that would fall under the generic test guideline ("me-too" methods). Another issue for PTMs is having quality standards to ensure consistency of the method over time. The PTM subcommittee asked ICCVAM to develop performance standards for three validated *in vitro* assays for dermal corrosivity (Corrositex®, EPISKIN™/EPIDERM™, and the rat skin Transcutaneous Electrical Resistance or TER assay). The EPA Scientific Advisory Panel (SAP) reviewed the performance standards because the SAP peer reviews specific decisions about pesticides and also because the performance standards were developed by ICCVAM after the initial validation.

Dr. Rispin said the SAP strongly endorsed the performance standards. She identified three key components of a performance standard. First, performance standards identify *essential test method components* that are the essential structural, functional, and procedural elements of a validated test method that should be included in the protocol of a proposed mechanistically and functionally similar test method. Second, performance standards include a *minimum list of Reference Chemicals* that should be used to assess the accuracy and reliability of similar test methods. Reference chemicals are well characterized chemicals that have been tested *in vivo* and in the *in vitro* or replacement system. Third, performance standards provide statistical standards of *comparable accuracy and reliability values* that should be achieved by a proposed test method when evaluated using the minimum set of Reference Chemicals. Dr. Rispin said that even though TER (which uses *ex vivo* discs of rat skin) is not really proprietary, it was

included because it is used by different countries with different laboratory setups. Thus, a generic test guideline would be useful.

The SAP concluded that the performance standards for each of the three *in vitro* methods were well described by ICCVAM and that the information should provide a basis to determine whether a test is mechanistically and functionally similar to a validated *in vitro* test method. An outcome of using well characterized and defined reference chemicals is that the original test often performs better on the minimum list of reference chemicals than the original entire database. This can be attributed, in part, to the fact that reference chemicals tend not to be compounds that give "borderline" results. She presented the generic criteria used by ICCVAM for selecting subsets of reference chemicals: 1) they should represent a range of chemical classes, 2) they should measure a range of corrosive strengths, 3) they must be well-defined (no mixtures) and available commercially, and 4) they must have unequivocal animal or other *in vivo* evidence.

The SAP made three recommendations on generic components of performance standards. First, they should state the minimum number of reference chemicals, which should be diverse and represent relevant chemical classes. Second, the list of minimum reference chemicals should represent different potencies or range of response, ideally within a chemical class. Third, the performance standards should include minimum standards for reliability and accuracy/concordance expected from the "me-too" test system's results when compared to the known properties of the *in vivo* tests. Dr. Rispin said some members of the SAP thought that the entire reference chemical database should be used for validating "me-too" studies. The agencies represented on ICCVAM felt this may be excessive. The SAP said it would be important to strike a balance between a manageable number of reference chemicals and assuring that all relevant mechanistic and chemical classes are included. Dr. Rispin concluded her talk by presenting three recommendations from the SAP to ensure consistent quality and test performance.

- Benchmark controls as well as positive and negative controls, should be tested in each new lot to determine the viability and usability of each lot.
- Benchmark controls are an important mechanism to assess both the adequacy of the method, as well as lot-to-lot variability, and should be considered as a standard component of these test methods.
- Benchmark controls should include several "classic" responders from different chemical classes/modes of action.

Discussion

Dr. Flournoy commented that she thought there should be some clarification on how accuracy and reliability would be assessed. Dr. Rispin responded that statistics of performance (sensitivity, specificity, false positives, false negatives, etc.) are conducted for the chemicals in the original validated prototype. These same statistical analyses are conducted for the subgroup of minimum reference chemicals. The "me-too" test method must perform in accordance with the generic guideline, tested in the minimum list of reference chemicals, and yield similar results. If the response is too variable, then the test method may not be a "me too", but rather a method that should be validated on its own. Dr. Fournoy replied that with a large number of statistical comparisons, there should be more discussion to the exact statistical procedure used to make these comparisons. Dr. Monteiro-Riviere appreciated the attention to quality control since she has observed significant inter- and intra- laboratory variability in her own and other laboratories. Dr. Monteiro-Riviere suggested that some of this variability could

be due to differences between chemical lots. Dr. Rispin said the SAP report included more information on quality control and alludes to the use of Good Laboratory Practices (GLP). The SAP also discussed the need to signal GLP auditors to look for performance in various ways and recommended that the lot-to-lot testing should take place with a sufficiently broad range of reference compounds. Although this would be a cumbersome process for GLP auditors to assess proprietary quality control procedures, the performance standards are expected to bring these issues out into the open. Dr. Goldberg asked if it were true that any compound applied to the skin must be tested *in vitro* in the United States as is the case for the European Union. Dr. Rispin said that the United States doesn't have such a statutory requirement, but one would expect that publication of guidelines with clear references to validated *in vitro* methods could, in practice, have the same effect as a statutory requirement. Dr. Rispin also said that in some cases, the requirements do not require testing for certain types of compounds, such as those in the extreme pH range; they are simply classified as corrosives.

Dr. Stitzel commented that the development of a mouse skin sensitization test method should lower the number of guinea pigs used in skin sensitization studies. She said tracking the guinea pig numbers should give one indication of whether efforts to promote alternative test methods are working. Dr. Rispin closed the discussion by commenting that the SAP recommended that a battery of reference chemicals with easily interpreted *in vivo* results should be used as a standard for development of many of these tests. This approach would allow for a more direct comparison of Corrositex, TER and EPIDERM™/EPISKIN™.

IX. Validation of Genetically Modified Mouse Models (GMM)

Dr. Bucher, NIEHS, presented SACATM with an overview on the use and evaluation of GMMs for cancer hazard identification at NIEHS/NTP. He began his presentation by saying that numerous recommendations have been submitted in public comments over the past several years indicating that NIEHS/NTP consider submission of GMM as a formal validation review of GMM.

Dr. Bucher identified four reasons for using GMMs for cancer hazard identification.

- Compared to the traditional two-year bioassay, GMMs result in a reduction of animal use in each group (50 versus 15).
- GMMs studies are shorter, six to nine months long compared to two years.
- The cost of GMMs is about one-third to one-half of the traditional mouse study.
- GMMs can potentially provide mechanistic information because these models exploit metabolic alterations in pathways involved in oncogenesis.

Dr. Bucher focused his presentation on three primary models and identified key NIEHS staff working on these models [Tg.AC (Dr. Raymond Tennant), p53 (Dr. Jef French), and Ha-ras2 (Dr. Bob Maronpot)]. Two of the models, Tg.AC and Ha-ras2 are ras-derived models; they use an activated "ras" gene to promote tumor development. Tg.AC is a □- globin promoter driven "v-Ha-ras" gene used as skin tumor model. Tg.AC also responds in the forestomach to some chemicals given orally. The p53 is a knockout mouse that has lost one of the p53 alleles and is purported to detect genotoxic carcinogens by mutation of the remaining p53 allele. Ha-ras2 is proposed to act through an over-expression of the ras gene (it contains multiple copies of the human "Ha-ras" gene). Of the three, the p53 model is the most understood.

Dr. Bucher briefly described the history of the use of transgenics for cancer hazard identification at the NTP. Beginning in the mid-1990's, Dr. William Eastin conducted several studies with Tg.AC and p53 to see if these assays could be adapted for use in a standard contract laboratory. Dr. June Dunnick also did some work with the p53 model that led to several changes in drug labeling (methylphenidate) and the removal of phenolphthalein from the market.

Over the past 6 years NTP/NIEHS has organized five formal reviews on the use of transgenics for cancer hazard identification.

"Review #1"
In 1998, the NTP Board of Scientific Counselors reviewed the p53 and Tg.AC models. The Board had conditional acceptance of the methods; they had little hesitation in accepting p53 data, but questioned the Tg.AC model. Overall, the Tg.AC model is burdened by not understanding exactly how the positive papilloma responses relate to carcinogenesis. Dr. Bucher said the Board was concerned by the lack of dose-response information and the lack of an understanding why some chemicals were negative in these studies, but were positive in the traditional two-year bioassay. The Board urged development of specific tumor-site models and a continued effort on these and other models as general carcinogen screens. Dr. Hayes asked Dr. Bucher to explain what "conditional acceptance" meant. Dr. Bucher said the Board did not think the studies were ready for routine use at that time, but recommended that work proceed to develop a database of general carcinogen screens that could then be the basis for a future evaluation.

Dr. Bucher said over 100 studies of various GMM cancer models have been sponsored by NIEHS/NTP, conducted either in-house or by contract laboratories. These studies include prevention of site-specific cancer, retrospective studies/model development (focusing on chemicals known to be carcinogenic in the two-year bioassay), and prospective studies (transgenic study conducted prior to the two-year bioassay).

In 2000, Dr. John Pritchard and other NIEHS staff began an evaluation of the concordance of selected transgenic mouse models (based on data from the program and the literature) with carcinogens listed by the International Agency for Research on Cancer (IARC) and in the Report on Carcinogens (ROC). Twelve different scenarios involving individual transgenic assays, combinations of transgenic assays, the two-year rat bioassay, and combinations of the rat bioassay with transgenic or genotoxicity assays were assessed. Overall, these approaches had an accuracy of 70 to 85 percent and the transgenic models alone did not perform badly. Dr. Bucher then presented the outcomes of an additional analysis to understand why accuracy was not 100 percent. He said the rodent two-year bioassays have missed calls, because they show positive findings for chemicals that the scientific community does not consider to be true human carcinogens ("over-calling"). He said a troubling outcome for the transgenic models is that there are instances where the transgenics are negative for probable or known human carcinogens. The overall conclusions from this review were that 1) transgenics are not overly sensitive models that will screen positive for every chemical and 2) transgenics are missing some probable and known human carcinogens. Based on these conclusions, NTP decided that positive finding from transgenics should be taken seriously, but that a negative should result in additional analysis.

"Review #2"

Dr. Bucher summarized the second review of transgenics by the NTP Board of Scientific Counselors Technical Reports Subcommittee in September 2002. The Subcommittee was presented with a review of Tg.AC dermal exposure studies for pentaerythritol triacrylate and trimethylolpropane triacrylate (both studies were positive). The Subcommittee was asked whether there was sufficient scientific evidence using this model to evaluate the potential carcinogenicity of each compound. The subcommittee rejected the proposed conclusion of "clear evidence of carcinogenic activity" and suggested that it would be more appropriate to develop model-specific descriptive language.

"Review #3"

The entire Board of Scientific Counselors was also presented with the issue of the transgenic knockout mouse development program in September 2002. Five questions were posed to the Board:

- Does the Board have recommendations regarding the issues to consider 1) in choosing a transgenic animal for mechanistic research and 2) in validating its use for screening?
- Under what conditions would the Board feel a positive result in a single or in multiple transgenic models sufficiently reflects a reasonable concern for carcinogenicity in humans? What additional research is needed to "validate" that the conditions suggested by the Board are scientifically sound?
- Under what conditions would the Board feel a negative result in a single or in multiple transgenic models sufficiently reflects little or no concern for carcinogenicity in humans? What additional research is needed to "validate" that the conditions suggested by the Board are scientifically sound?
- Does the Board have suggestions concerning research the NTP can support to determine if positive findings in transgenic models can be used to predict risk (level of exposure versus probability of carcinogenic response) in human populations?
- To what degree would the Board suggest that we balance further research on the development of transgenic animals for understanding mechanisms with the validation of these animals as part of a carcinogenicity screening program?

Dr. Bucher said that although the Board worked very hard, they were unable provide clear answers to the questions.

"Review #4"

Dr. Bucher summarized the outcome of a workshop the NTP held in February 2003 to address the recommendation by the NTP Board of Scientific Counselors Technical Reports Review Subcommittee to develop interpretative language that would properly communicate the results of transgenic studies to the scientific community. Two questions were presented at the workshop.

- Does the scientific/regulatory community consider tumor findings in genetically modified mouse models as equivalent to tumor findings in traditional rodent cancer models? Is the answer the same for all commonly used models (Tg.AC, p53+/-, rasH2)?
- To what degree is the scientific/regulatory community confident that negative results in studies with genetically modified mouse models are equivalent to negative results in the traditional bioassay?

To address the workshop charge and discussion questions, a dozen case studies were presented that identified the model, study parameters, tumor incidence, statistical strength of response and historical tumor rates. Workshop participants were asked to vote on the findings by using the descriptions for two-year bioassay outcomes (clear evidence, some evidence, equivocal evidence, or inadequate study). In response to the first question, workshop participants concluded that comparing tumor findings in GMMs to traditional mouse cancer models could only be done on a case by case basis, that strong responses may be similar and that negative responses may not be similar. Participants also gave a qualified recommendation that NTP continue to conduct p53 +/- or rasH2 studies, but only on a case-by-case basis should these models be used place of the B6C3F1 mouse study. Dr. Bucher said there was no clear answer to the second question.

"Review #5"

Dr. Bucher presented SACATM with the outcome of the most recent review of transgenics by the NTP Board of Scientific Counselors Technical Reports Subcommittee (May 2003). The Board was presented with a review of Tg.AC, p 53+/- and p16/19+/- studies of aspartame and Tg.AC and p53+/- studies of acesulfame K (both chemicals are non-nutritive sweeteners). The Subcommittee accepted the conclusions from the p53 study for "no evidence of carcinogenic activity," but had more complicated interpretations of the Tg.AC and the p16/19+/- results[3].

These studies were presented to the Board in the form of a new technical report series labeled the Genetically Modified Mouse Model (GMM) series. Dr. Bucher presented the forward to this series:

> These studies are designed and conducted to characterize the toxicologic potential, including carcinogenic activity, of selected agents in laboratory animals that have been genetically modified. These genetic modifications may involve inactivation of selected tumor suppressor functions or activation of oncogenes that are commonly observed in human cancers. This may result in a rapid onset of cancer in the genetically modified animal when exposure is to agents that act directly or indirectly on the affected pathway. An absence of carcinogenic response may reflect either an absence of carcinogenic potential of the agent or that the selected model does not harbor the appropriate genetic modification to reduce tumor latency and allow detection of carcinogenic activity under the conditions of these subchronic studies.

Validation Issues

Dr. Bucher presented SACATM with three validation issues that arise when considering whether to present a GMM submission to ICCVAM:

- Can operational characteristics (such as intra- and inter-lab reproducibility, relevance – sensitivity/specificity, and study limitations) be determined?

[3] For Tg.Ac, the Subcommittee accepted a conclusion of "no evidence of positive response for papilloma formation in the forestomach or for tumors at other sites in male or female Tg.AC mice administered aspartame/acesulfame K in feed at concentrations up to 50,000 ppm for 9 months." The conclusion based on the p16/19 model was "no evidence of enhanced tumor formation in a p16/19 tumor suppressor mouse model; this model is currently uncharacterized in terms of its expected tumor response to known rodent and/or human carcinogens and noncarcinogens".

- Second, what is the gold standard, human carcinogens, rodent carcinogens or a combination of the two?
- Is it possible to use "mechanisms" in a validation exercise? For example, should studies be conducted to verify the involvement of a transgene or knockout as part of the mechanism leading to tumor production?

Validation/Evaluation

Dr. Bucher closed his presentation by asking SACATM to comment on several questions related to validation and evaluation:

- Should NICEATM be tasked with extending the Pritchard *et al.* analysis (to examine factors such as the comparability of protocols, the impact of modifying these protocols, consistency in study performance, criteria for evaluating studies, use of GLP, sufficiency of replicate experiments, adequacy of the model for the chemicals study, and animal welfare consideration)?
- Is it appropriate for ICCVAM to use its evaluation process to review the scientific validity of these transgenic mouse models?
- What are the appropriate reference test systems or reference data that should be used to assess the predictiveness of these test systems?
- How might information on mechanism be used in the validation process?

Public Comment

Dr. Richard Becker presented public comments of behalf of the American Chemistry Council (ACC). First, he said validation is needed for these models and ICCVAM is required by law to be the lead in validation of new and alternative test methods in the United States. He clarified that he was not trying to dismiss the tremendous amount of work that has already been done, but the ICCVAM validation review process would provide answers to unanswered questions of how these test methods could be used for regulatory risk management purposes. Dr. Becker then presented dose-response data for a few chemicals tested in Tg.AC models. His conclusion from these data is that the model should not be used for substances, or at doses of substances, that produce skin irritation or damage. He also thought the model provided qualitative information, but that the data should not be used to extrapolate qualitatively or quantitatively to humans. He recommended that a nomination to initiate a full validation review be presented to ICCVAM.

Martha Marapese, a partner with Keller & Heckman, presented public comments on behalf of RadTech International North America. RadTech is a non-profit organization consisting primarily of small businesses that utilize ultraviolet (UV) and electron beam (EB) technology to coat a variety of products. Ms. Marapese presented public comments because NTP is moving forward with two-year dermal studies on two substances that can be components of UV and EB curable coatings [trimethyl propane triacrylate (TMPTA) and pentachloroerythritol triacrylate (PETA)]. Further, these two-year studies are planned for validation purposes for the Tg.AC assay. RadTech had two primary concerns about these test. First, RadTech is concerned about the selection of TMPTA and PETA as test compounds given that industry is working voluntarily with EPA to address data needs. Second, RadTech is concerned that the doses NTP is proposing to test include dose levels that would be expected to cause skin irritation, which would make interpretation more difficult. If NTP moves forward with these studies, then RadTech requested that the use of data generated by these studies be restricted to the purpose of validating the Tg.AC model.

Sara Amundson, Doris Day Animal League, said Dr. Bucher's presentation was very interesting. She raised five main points during her public comment. First, she said it doesn't matter if a test is an alternative, revised or new. Federal regulatory agencies cannot require or recommend a new test method within those parameters without ensuring that it has been appropriately scientifically validated. Second, Ms. Amundson was concerned that many of the materials coming out of ICCVAM and SACATM focus on the validation of alternatives, rather than developing new and revised test methods. Third, she was pleased that many concerns about transgenics models raised by the animal protection community have been heard. They do not consider these tests to be alternatives, but rather new and revised methods. Fourth, she said there is a solid definition of test validation in the ICCVAM Act and it really has to be a process that is addressed by the ICCVAM. Finally, she was concerned about the large number of animals utilized in order to develop GMMs. Because these animals are mice, they fall outside the purview of the Animal Welfare Act.

Discussion

Dr. Hamernik, EPA, said the regulatory applicability of transgenic methods needs to be considered when deciding whether to proceed with a validation effort for transgenic methods. She did not think these methods would be useful now without some sort of supplementation. Dr. Hamernik raised some general concerns about dose-response relationships, potential metabolic differences between transgenics and the strains typically used now, and whether these models would be expected to detect carcinogens that act by indirect mechanisms such as those involved in the endocrine system.

Dr. Portier reminded SACATM of the two general questions posed to them. One is whether NTP should proceed with a validation of transgenics. The other pertains to the question of whether current validation procedures take into account the complex issues raised by GMMs. Dr. Portier explained that in both the IARC and ROC processes, identification of hazard is not dependent on a single assay, but is based on the strength of evidence. It is unclear how validation moves forward with mechanistic assays that may play a role in strength of evidence approaches for hazard identification. For transgenic models, one approach may be to re-review IARC and RoC decisions in light of the removal of a particular assay. Another approach could be to see how one assay predicts overall evaluation. However, since the calls by IARC and NTP are based upon positive findings in two species, comparing one assay against a call that requires two studies is not quite fair. Dr. Portier also said that in some cases transgenics were developed to address issues of mouse liver carcinogenicity related to relevance of mechanism to human carcinogenicity.

Dr. Safe, one of four lead discussants for this topic, said he did not think NTP should proceed with validation of transgenic methods because they are not a suitable model. Any information obtained from animals is applicable only to that animal and not to normal mice because they contain an oncogenic or tumor suppressor mutation. He believed that the transgenic models are good for mechanistic and chemotherapy studies, but not for testing a carcinogen in an animal with a background oncogenic equivalent. Dr. Theran, another lead discussant, commented that a mouse is not a human, so should the genetically modified mouse not be used because it's not a mouse, or should the issue be whether it may detect cancer compared to the human experience? Dr. Safe agreed that the question of what is a good model for humans is always an issue, but that a genetically modified mouse is a worse model than the rat or mouse used currently.

Dr. Goldberg, another lead discussant, raised several points. First, he said that transgenic models present a couple of unique animal welfare concerns. Often these animals are isolated, so there are enrichment issues and the outcome of the transgenesis may result in models that are compromised. Second, he considered transgenics to be an alternative test method since they offer the opportunity to reduce animal numbers and distress. Third, the mechanistic concern is that the models will miss compounds that produce tumors by mechanisms other than the one inserted into the animals. However, the definition of validation is the use of a test for a specific purpose and perhaps the strategy should be to not over-define the purpose of the test. Third, the use of transgenics in combination with non-invasive approaches, such as MRI or PET scanning, can potentially reduce animal use. These methods also can reduce pain to the animal because they can potentially detect a tumor at the earliest stages. Dr. Goldberg concluded by saying that he thought transgenics offer a real opportunity that should be carefully explored.

Dr. Willhite, a lead discussant, quoted from several of the peer-reviewed papers listed as background material that led him to conclude overall that 1) the use of transgenics may not decrease total animal use for carcinogenicity testing, 2) these models are not ready for validation, and 3) they are not sufficiently robust for human health assessment. Some of the points raised by Dr. Willhite to support his position were comments in the papers about the need for a battery of transgenic models, increased group size numbers, and the use of wild-type mice in control and high doses. He felt these models are useful for investigating mechanistic issues, but there is a need for more universal and highly sensitive models. Dr. Poland, NCI, also did not think the transgenic models are appropriate in the current context. He said the standard two-year bioassay is really for complicated chemicals, because industry does not develop compounds that screen positive for mutagenesis. Dr. Wind, CPSC, did not support having ICCVAM validate these studies. She said since NTP made it very clear that they do not believe the mouse model should be replaced by transgenics, there would be no point for ICCVAM to spend money to validate a model that is not going to replace, refine, or reduce the use of animals. Dr. Stitzel agreed with Dr. Wind and said this was a program that has gone on too long. However, she felt that Dr. Portier raised a very good point of how to deal with an assay that may be valid for only certain things. Dr. Stitzel did not feel such an assay, which would be very expensive to validate, should take priority over other test methods because it would have no regulatory applicability. Dr. Dean commented that the pharmaceutical industry had a major, very expensive effort through HESI to validate these assays and the conclusion was that they are unsuccessful as a replacement. Dr. Portier told the committee that the primary focus in this discussion should not be whether to validate these methods through ICCVAM. The comments of SACATM along with other groups did not indicate support for this effort. Nevertheless, the issue of transgenic models will continue to arise (such as for use as a screen) and the real issue is whether the existing paradigm can accommodate complicated validation issues.

Dr. Theran did not voice all of his points since most had already been raised by others. He did say that he had concerns about the ability of institutions that manage large colonies of transgenic animals to care properly for the animals they create. For example, he said in one case, a mouse model developed ocular discharge as a phenotype, but that the institution did not have the resources to clean the eyes on a regular basis.

Dr. Dean asked Dr. Portier to clarify his question because he was unsure why there would be outstanding questions about how to validate these models if the committee felt they had no

utility. Dr. Portier responded that hazard interpretation is not necessarily based on a single assay. The goal would not necessarily be to replace one assay, but to change the nature of the data used for the weight-of-evidence evaluation. Dr. Curren said in order to do a weight-of-evidence evaluation for a multi-factor problem, one would need to know the contribution, relevance and reliability of each factor. Dr. Curren cautioned against developing a battery where even though each assay is contributing to prediction, the overall accuracy of the battery never becomes acceptable. Dr. Curren felt that it could be reasonable for ICCVAM to consider doing a validation evaluation of transgenics depending on what the proposed purpose would be.

Dr. Hayes asked for clarification on whether Dr. Portier was suggesting that a transgenic study be conducted in addition to the two bioassays used now and what would be the argument for maintaining the transgenics. Dr. Portier responded that the NTP is not trying to maintain transgenics, but rather address the larger question of validation issues when one assay is not simply being replaced with another. For example, it could be 15 studies would replace one or two. Dr. Stitzel agreed with the points raised by Dr. Curren, and she agreed with Dr. Portier that it is important that a strategy is developed to move beyond the paradigm of one test replacing one test. She said developing prediction models for specific transgenic assays would be the appropriate review for ICCVAM, not for ICCVAM to conduct a review of all transgenic mice for carcinogenicity. Dr. Sonnenshein suggested waiting before making a decision on this issue because the answer is not clear. Dr. Safe extended Dr. Sonnenshein's response to say that since the answers are unclear, the recommendation should be not to proceed with validation efforts. He added that there are better models than p53 and ras, especially conditional knockouts. Dr. Dean asked Dr. Portier whether there is a need to poll the committee. Dr. Portier responded that there was not, the advice was pretty clear. He asked the Strategic Planning working group to consider further the issue of where validation is going in the next few years.

Dr. Becker asked for clarification on whether the recommendation to not move forward with validation of these models includes a recommendation to not use these models. Dr. Bucher responded that NTP began scaling back on the use of transgenic in terms of putting new test compounds into transgenic animals. Dr. Bucher said it was not the case that these models are uniformly interpreted as having no utility. For example, the FDA Center for Drug Evaluation and Research (CDER) accepts transgenic animals as part of their drug registration. This test is not required, but a transgenic mouse can be used in conjunction with the rat. Dr. Jacobs, FDA CDER, said that an agreement was reached at the International Conference on Harmonization a number of years ago concerning carcinogenicity testing in pharmaceuticals. It was agreed internationally that carcinogenicity testing could be done in rats and in a scientifically valid, but unspecified, alternative model. In the past eight years, about 25% of carcinogenicity tests have been conducted in alternative models [transgenics] and these have been accepted in place of the traditional mouse bioassay. Dr. Stitzel asked what the FDA would do if ICCVAM looked into validating these models and concluded that they could not be validated. Dr. Jacobs replied that ILSI had collected significant amounts of data and the conclusion was that they were appropriate enough for use. The p53 is only acceptable if a compound is equivocal or positive for genotoxicity, but the p53 is inappropriate if a compound is not genotoxic, because it is not expected to detect carcinogens that act through indirect mechanisms of carcinogenicity. The Tg.AC and H2ras can be used for either genotoxic or non-genotoxic chemicals. Dr. Sonnenshein asked for clarification on whether a mutagenic drug is necessarily considered to be carcinogenic. Dr. Jacobs replied that the conclusion from the ILSI data is that the p53 model detects genotoxic human carcinogens. Dr. Dean asked if this means data being submitted on non-validated methods are in violation of the ICCVAM Authorization Act. Dr. Jacobs said the

FDA can accept methods that have been scientifically validated; a formal validation is not required. Dr. Portier emphasized that each agency can decide independently what methods are considered valid, but an ICCVAM review is the mechanism to establish a method as valid across all Federal agencies. Dr. Curren said that while some on the panel felt that transgenics are not an appropriate candidate for an evaluation of validity, he would not necessarily consider this a panel conclusion. He would support an ICCVAM evaluation of the validity of specific transgenics if a prediction model said that a certain GMM is very good at predicting a certain endpoint or piece of toxicological information.

Dr. Green asked if any of the other agencies use transgenic models in a weight-of-evidence evaluation. Dr. Hatten, FDA Center for Food Safety, said his center did not feel that GMMs are particularly useful for fulfilling their regulatory mandate. For example, in contact materials for a food wrap there may be inadvertent exposure to very low levels of a carcinogen. In this case, the exposure to the carcinogen may be allowed if there is a sufficient margin of exposure. Transgenic models do not provide estimates of cancer potency. Dr. Stitzel commented that it sounded like the FDA CDER is using the transgenics for a specific purpose and not just as a replacement. Dr. Jacobs said the alternative is a replacement for the traditional mouse assay. A drug sponsor can submit any alternative with data supporting scientific validity of the method. Dr. Goldberg said construction of building facilities for transgenics confirmed what Dr. Jacobs said; these facilities are being built by universities and pharmaceutical companies, but not by the pesticide or chemical industry. Dr. Dean said at his company, transgenics are used as disease models and he is unaware of companies routinely using them as carcinogenicity screens. Dr. Poland said at some point in the future it may be possible that a transgenic mouse could be used in conjunction with other data, like gene array data, to reveal a pattern of response that raises concern.

Ms. Amundson, Doris Day Animal League, said the FDA CDER is not in violation of the ICCVAM Act because this decision transpired eight years ago. However, now as Federal agencies move forward in considering new, revised or alternative methods, they will need to carefully abide by a definition of validation that pertains to the three R's. Dr. Portier noted that FDA's decision on a particular drug is not based on one test. He said this is an example that illustrates the need to address complex validation issues. Dr. Goldberg asked Ms. Amundson to clarify what she meant when she said that a Federal agency would be in violation of the ICCVAM Act if it used a test that has not gone through a formal validation process as described by ICCVAM. Ms. Amundson said the issue is still open to interpretation, but the issue is one of Congressional intent. The statute contains a clear definition of validation and there is also a clear reference to agency action to ensure that any new, revised or alternative test method recommended or incorporated into their regulations has been deemed scientifically valid. When these two sections are combined, there is Congressional intent. Dr. Stitzel said it sounded like there is a difference between a company providing data and an Agency requiring, encouraging or recommending a method.

Dr. Dean adjourned the first day of the meeting at 5:35 p.m.

March 11, 2004

Dr. Flournoy, Acting Chair, welcomed everyone to the second day of the SACATM meeting. She asked people seated at the table and observers in the room to introduce themselves. Dr. Thayer read the conflict-of-interest statement.

X. Update of ILSI/HESI Technical Committee's activities on identification of biomarkers of toxicity

Dr. Dean, reported on the activities of the ILSI-HESI Technical Committee on Development and Application of Biomarkers of Toxicity. The technical committee is interacting with ICCVAM in case any methods are developed in this process that could be validated and used for regulatory decision making. Dr. Dean is chair of this committee. He explained that the Health and Environmental Sciences Institute (HESI) is an international branch within the ILSI organization. While ILSI is an organization primarily focused on food and food safety, HESI addresses the issues of four primary industries: agricultural chemical, chemical, consumer product and pharmaceutical companies. Dr. Dean explained that an Emerging Issues Committee polls these organizations for input on technical or scientific issues involved in risk assessment and toxicology that need to be addressed. Committees composed of representatives from academia, industry and government develop approaches to address the identified issues. ILSI is a non-profit group funded mostly by industry; the Board of Trustees is split between academics (public) and industry representatives.

The mission of the ILSI HESI Biomarkers Technical Committee is:

> To advance the scientific basis for the development and application of biomarkers of target organ toxicity; to develop a systematic approach based on newly available technologies for the identification of biomarkers that bridge from the pre-clinical to clinical stages of drug development; and to provide a scientific forum for building consensus regarding how to apply biomarkers of toxicity in risk assessment.

Dr. Dean said the biomarkers should have several characteristics. They should bridge the pre-clinical and clinical stages of drug development, be non-invasive, and they should leak into some body fluid where they can be measured. The working group is also trying to build consensus around whether these biomarkers have any utility in the risk assessment process.

Dr. Dean presented the history of the Technical Biomarkers Committee. A subcommittee was formed in January 2002 following selection of the topic as a top emerging issue by the ILSI-HESI membership. The subcommittee held its first exploratory meeting in November 2002 and a project was proposed to solicit biomarker candidates. Between April and June 2003, seven proposals were received. Three proposals were selected for evaluation: biomarkers for nephrotoxicity, serum cardiac troponins and inhibin B for testicular toxicity. Three expert working groups (EWGs) were formed to address these proposals. In January 2004, the HESI Board of Directors approved full technical committee status for the subcommittee and fully endorsed the project[4]. The project is currently in the assay development phase, where analytical methods are being developed for biomarker evaluation protocols. Following the assay development stage, there will be an interim evaluation reviewing in-life study feasibility and cost. Biomarkers selected for further evaluation will proceed to an in-life protocol for testing in multiple laboratories (up to one year). At this stage, the focus will be on assay sensitivity, specificity, reproducibility, and predictive value. The results of the evaluation will be published in the peer-reviewed literature, and as appropriate, submitted to ICCVAM for review. The

[4] After the SACATM meeting, Dr. Dean provided the name and contact of the committee coordinator: Dr. Amy Lavin at HESI; Email: alavin@ilsi.org.

subcommittee has been consulting with ICCVAM along the way; Drs. Hamernik, Schechtman, and Stokes are ICCVAM liaisons to the subcommittee.

Biomarkers of nephrotoxicity
Dr. Dean identified five biomarkers of nephrotoxicity to be evaluated. Some of these biomarkers are used in clinical transplantation medicine as early indicators of kidney rejection.

- □ glutathione-s-transferase (GST) – marker for proximal tubule damage
- Kim1 - marker for proximal tubule damage
- □ GST – marker for distal tubule damage
- PAP1 – a papilla marker
- clusterin – a general marker of nephrotoxicity

Evaluation of these biomarkers is currently in the assay validation and development phase. Pilot studies are planned to look at the stability of these markers in urine and evaluate the detection limits of the assay systems. ICCVAM has been consulted on the protocol for a GLP study planned across at least three sites. The EWG is currently working to identify reference toxicants.

Serum Cardiac Troponins
Dr. Dean explained that troponin has been used as an early indicator of myocyte injury in humans and the committee believes it could be easily expanded to other species. Changes in troponin levels correlate with the development of drug-related cardiotoxicity and ischemia. There are three troponins (T, I, C) located among actin filaments in cardiac muscle. The troponins leak upon damage or ischemia to the muscle filaments. Troponin I is associated with ischemia and troponin T is believed to reflect more general tissue damage. Dr. Dean outlined several potential research goals, including evaluation of the kinetics of release and return to baseline, identification of the diagnostic advantage of I/T, and assessment of whether there is a threshold for an increase in serum troponin below which there is no evidence of cardiac injury. He presented several components of the testing paradigm, including planned studies in rats, dogs and monkeys, and assessment of troponin detection using commercial kits.

Inhibin B as a biomarker of testicular toxicity
Dr. Dean reviewed the basics of inhibin B regulation in males. Follicle stimulating hormone (FSH) and luteinizing hormone (LH) released from the pituitary gland act on testicular Sertoli cells to stimulate the production of inhibin B. Inhibin B in turn feeds back to decrease FSH secretion. Inhibin B is already used to monitor reproductive function in man and may be a more sensitive marker of function than variables like sperm count and sperm quality.

Preliminary phase studies for inhibin B are planned that will 1) examine cross-reactivity of the commercially available kits for different animal species, 2) examine the analytical variability of the assay, and 3) establish normal reference ranges for SD and Wistar rats. Test compounds have not been finalized, but will be selected to target various cell types within the testes. The second phase of this study will use standardized protocols that cover a full-dose response. Necropsies will occur during early, peak and recovery phases and include histopathology, hormone measurement (FSH, LH, testosterone, and dihydrotestosterone), sperm counts/functional evaluation, and organ weight, in addition to inhibin B levels.

The anticipated impact of developing these biomarkers would be for safer, faster, less costly drug development, and hopefully animal reduction. Dr. Dean also noted that these biomarkers may have applications in surveillance of populations that have some suspicion of environmental exposure to toxicants. There are 18 companies involved in this effort representing most of the pharmaceutical industry. In January 2004 at the ILSI-HESI annual meeting, this project was advanced to full technical status which means the companies now have to contribute support to the effort. Representatives from the private and government sector have been invited to attend the expert working groups to help guide the subcommittee in the right direction.

Discussion

Dr. Willhite suggested that the committee keep organizations like the National Institute for Occupational Safety and Health (NIOSH) appraised of the testicular toxicity project. Dr. Dean responded that the outcome will be published in the peer-reviewed literature. Dr. Green asked what the nature of ICCVAM's input is because he is concerned that a considerable number of groups developing test methods may want similar input from ICCVAM, which might overwhelm ICCVAM's resources. Dr. Dean explained that the input is more advisory at this point, such as asking for input on whether this is a design that may be eventually evaluated. Dr. Schechtman added that ICCVAM's efforts would increase when the review process begins, but the hope is that the review process can be smoother if ICCVAM guides organizations to develop complete packages for submission. Dr. Schechtman agreed that ICCVAM will need more resources as the number of submissions to ICCVAM increase since ICCVAM representatives and NICEATM staff are already overwhelmed. Dr. Dean said he worries that without the ICCVAM consultation, it would be possible to use animals and invest resources to develop a method that could not be validated. Dr. Dean also said he feels that the consultation could make the review process faster and with less resources used on both sides. Dr. Stokes elaborated that NICEATM has always communicated with test developers in preparations of submissions. He added that a complete submission allows for quicker organization of a peer-review panel, evaluation by ICCVAM-NICEATM and recommendations going to agencies.

Dr. Portier raised three points. First, he noted that some of the proteins presented are broad spectrum and he wondered how the subcommittee will address issues like false positives from an organ that might be stimulated to produce the same protein. Second, some of the biomarkers have polymorphisms that may make it difficult to compare animals and humans. Third, Dr. Portier asked to what degree the committee is exploring cell-based assays for the same purposes. Dr. Dean responded that with respect to the false positive issue, they will run negative compounds to make sure they are not getting the wrong signal and do comparative histopathology. He said the committee is addressing polymorphism between the rat and human early in the process. In response to the third point, Dr. Dean said metabolism and absorption issues make it difficult to rely on an *in vitro* system early in the drug development process. Dr. Stephens asked for clarification of the three R's relevant to biomarkers. He also asked if use of these assays early on would preclude downstream testing or would they only be used in cases of suspected toxicants? Dr. Stephens said he would not like to see these assays be added for every drug and only occasionally prevent downstream testing. Dr. Dean replied that these assays would be used in the pre-clinical situation, and potentially in discovery, to cull candidates that have potential toxicity. He believed these assays would, over time, decrease animal use and provide a bridge to the clinical situation. Dr. Goldberg suggested that these assays, once shown to be effective, might be modeled to *in vitro* systems for a specific endpoint. Dr. Dean said yes, and they may lend themselves to high throughput drug screening.

Dr. Clark, Xenobiotic Detection Systems, suggested that SACATM be used as the peer-review body instead of convening another peer-review. He added that a separate peer-review committee could be brought in if the work load became too much. Dr. Stokes replied that ICCVAM and NICEATM would consider ways to use SACATM, if appropriate, rather than convene a completely independent peer review panel. Dr. Hatten, FDA, outlined several reasons why time delays in the ICCVAM process might occur. First, the test method might not be in the state of development that the submitter thinks it is and the result is that the method cannot be accepted or validated. Second, the data may exist, but spread throughout the literature. If submitters follow the guidelines and gather the existing information in an appropriate format, then the process would go more quickly.

XI. ICCVAM Nominations

Dr. Stokes said he would review the ICCVAM nomination process and prioritization criteria, discuss nominations on endocrine disruptor test methods and *in vitro* ocular irritation test methods, and briefly discuss a pending test method nomination.

A. Overview of ICCVAM Nomination and Submission Process and Prioritization Criteria

Dr. Stokes said that the nomination and submission process is available in the ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods published September 2003 (these guidelines are also available on the ICCVAM-NICEATM website at http://iccvam.niehs.nih.gov/). Dr. Stokes distinguished between test method nominations and submissions. Submissions are more complete and include data and information for test methods that have been through validation studies and are ready for peer-review and consideration for regulatory acceptance following the peer-review. Nominations lack a complete test method submission package. For example, nominations may need additional validation efforts or a test method may be proposed for pre-validation or validation studies, or as a topic for a workshop. Following receipt of nominations, NICEATM conducts a preliminary evaluation to review the extent to which the nomination addresses ICCVAM prioritization criteria. After the NICEATM evaluation, the nomination goes to ICCVAM for review and a preliminary draft recommendation. Comments are sought on the draft recommendations and both the draft recommendation and comments are presented to SACATM. These comments go back to ICCVAM and ICCVAM finalizes its recommendations. NICEATM presents this to the director of the Environmental Toxicology Program (ETP) for funding consideration and if the resources are available, the activity begins.

Dr. Stokes presented the ICCVAM prioritization criteria:

- Extent to which the proposed test method is:
  - applicable to regulatory testing needs
  - applicable to multiple agencies/programs
  - warranted, based on the extent of expected use or application and impact on human, animal, or ecological health
- Potential for the proposed test method, compared to current test methods accepted by regulatory agencies, to refine, reduce or replace animal use
- Potential for the proposed test method to provide improved prediction of adverse health or environmental effects, compared to current test methods accepted by regulatory agencies

- The extent to which the test method provides other advantages compared to current methods (e.g. reduced cost and time to preform)
- The completeness of the nomination or submission with regard to ICCVAM test method submission guidelines

B. In Vitro Endocrine Disruptor Nominations

Dr. Stokes discussed two *in vitro* endocrine disruptor nominations. Both of these methods adhere to test method development recommendations put forth in the report "ICCVAM Evaluation of *In vitro* Test Methods for Detecting Potential Endocrine Disruptors." The two recommendations outlined in the report are that priority should be given to development of assays 1) that not require the use of animal tissue or surgical procedures and 2) that do not use radioactive materials. One test method nomination is a biosensor system that can assess estrogen receptor binding and transcriptional activation. Pre-validation studies on this method are expected by June and the developer intends to submit the data and request funding for a multi-laboratory validation study. The second method is a stably transfected recombinant cell-based transcriptional method. The developer has completed pre-validation studies for 120 chemicals. NICEATM has requested the results of these studies.

The two test method nominations fulfill ICCVAM Prioritization Criteria, with the exception of completeness of the submissions, which is to be determined. ICCVAM recommended that validation of these test methods receive a high priority for support, contingent on review and determination that the proposed validation studies adhere to recommendations in the report "ICCVAM Evaluation of *In vitro* Test Methods for Detecting Potential Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays." The next step is publication of a Federal Register notice soliciting comments on these methods and asking for other nominations of this nature. NICEATM will request that the sponsors provide pre-validation study results, the proposed standardized protocol for the studies, and the proposed validation study design. [The Federal Register notice was published on April 21, 2004]. An ICCVAM Working Group will review this information and develop draft recommendations for ICCVAM. The ICCVAM recommendations will be presented to SACATM. ICCVAM will consider the comments from SACATM and the public and prepare final recommendations. NICEATM will then request funding from the director of ETP.

C. In Vitro Ocular Irritation Test Methods

Dr. Stokes reminded SACATM that at the August 2003 meeting, EPA announced plans to nominate *in vitro* ocular toxicity tests to ICCVAM for evaluation. The EPA nomination emphasized test methods to identify severe irritants without animal testing. At the same meeting SACATM unanimously approved with high priority that ICCVAM and NICEATM review the validation status of these methods and carry out appropriate follow-up activities. A public comment was made at the August meeting that one of the four available *in vitro* ocular test methods is routinely used in-house by a large chemical company prior to animal testing. EPA submitted a nomination in October 2003 for four test method activities. First, EPA asked ICCVAM-NICEATM to conduct an evaluation of *in vitro* test methods that could serve as screens for severe (irreversible) ocular irritants or corrosives. These screens could eliminate the need to use animals to identify severe ocular irritants/corrosives. Second, EPA asked for a state of the science evaluation of *in vitro* test methods for assessing non-irritants, and mild or moderate (reversible) irritants. The third component of the EPA nomination was a request for ICCVAM to obtain existing and, if necessary, generate good quality *in vivo* eye irritation/corrosion reference data to assess interlaboratory variability and support the validation

of *in vitro* eye tests. Fourth, EPA asked that ICCVAM explore strategies to alleviate pain and suffering that might arise from exposure to mild and moderate irritants in the current *in vivo* eye irritation tests while such tests still need to be used. ICCVAM unanimously recommended that these four activities be conducted with high priority. The two activities with the highest priority were the review of test methods that can identify severe ocular irritants/corrosives and a review of existing *in vivo* data to identify appropriate reference chemicals for validation studies. ICCVAM re-established an ocular toxicity working group to review these nominations. A Federal Register notice was submitted for publication (published on March 24, 2004) requesting comments and submission of data relevant to the four activities in the EPA nomination. In addition, ICCVAM and NICEATM are coordinating with ECVAM to leverage resources and minimize duplication of effort.

Dr. Stokes summarized proposed activities for the four ocular toxicity activities nominated by EPA:

Nomination #1: In vitro Test Methods for Identifying Substances Causing Severe/Irreversible Ocular Damage

Dr. Stokes identified the four *in vitro* test methods under review: the Bovine Corneal Opacity and Permeability Assay (BCOP), the Hen's Egg Test on Chorioallantoic Membrane (HET-CAM), the Isolated Rabbit Eye test (IRE), and the Isolated Chicken Eye (ICE) Test Method. Considerable amounts of data exist for each of these methods. Dr. Stokes said they are accepted on a case-by-case basis by some European Union countries, but have not yet been adopted into European guidelines. ECVAM would like to have the ICCVAM validation review completed before recommending that these methods be incorporated into European guidelines. The Global Harmonized Scheme (GHS) for classification developed by the United Nations allows for use of validated and accepted *in vitro* methods to identify severe ocular irritants/corrosives without further testing. Dr. Stokes presented a figure of the testing and evaluation strategy from the GHS publication that showed that if a valid test for assessing severe ocular damage is available, then a positive result can be classified as a category 1 hazard. If the result is not positive, then testing proceeds into a tiered testing strategy.

Dr. Stokes said numerous studies have addressed the question of whether these methods are valid replacements for all ranges of severity of ocular irritation. None of the studies concluded that the *in vitro* methods are acceptable replacements. Dr. Stokes added that none of these studies evaluated the test methods for the specific proposed use of identifying severe irritants or corrosives. He highlighted several shortcomings of past evaluations: 1) the protocols for the same method varied among studies, 2) assessment of test method accuracy was based on correlation with *in vivo* mean average score (MAS) and different *in vivo* ocular irritation classification systems were used (although not the US or GHS classification scheme), and 3) individual animal data necessary to assess accuracy for predicting US and GHS hazard categories were not made publically available. ICCVAM will consider these issues in their evaluation; for example, NICEATM will contact study authors to obtain original *in vivo* and *in vitro* data and identify test substances. Dr. Stokes said the nest step would be to prepare background review documents for each of the four test methods. The review documents would identify essential test method components, standardized protocols, a list of reference substances with high quality *in vivo* ocular irritation data, and, if adequate data exist, develop proposed performance standards that screening or replacement *in vitro* ocular toxicity test methods should meet or exceed. An expert panel would be convened to review and comment on the Ocular Toxicity Working Group recommendations. Dr. Stokes presented a tentative

timeline for these activities. The background document is expected to be released in November 2004 followed by a meeting of the expert panel in January 2005. Publication of the final expert panel report and request for comments are expected for April 2005. By July 2005, ICCVAM will consider comments and publish final recommendations.

Nomination #2: Review of State-of-Science and Availability of In Vitro Test Methods for Assessing Moderate, Mild, or Non-Irritation

Dr. Stokes said several activities are underway for this nomination, including a literature search of relevant *in vitro* test methods and publication of a Federal Register notice requesting relevant data [published March 24, 2004]. ICCVAM and NICEATM are aware of 44 different assays that have been used; these assays have different levels of available data. ICCVAM will have to develop criteria to identify the most promising *in vitro* methods to move forward. ICCVAM will collaborate with ECVAM on these activities and as appropriate, convene a workshop, expert panel meeting, or peer panel meeting to review findings and recommendations.

Nomination #3: Obtain Existing and/or Generate Good Quality In Vivo Eye Irritation/Corrosion Reference Data

Dr. Stokes said NICEATM is currently evaluating the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) database that has 132 chemicals with individual raw data for ocular toxicity testing. He said a Federal Register notice will be published that will also request additional *in vivo* data. ICCVAM and NICEATM are collaborating with EPA to evaluate the TSCA database for ocular irritancy. This database contains records for over 2500 chemicals and NICEATM will identify those that are commercially available, have individual animal data, and can be considered as possible reference chemicals for validation studies. He said authors of published studies would be contacted for additional data and information. In addition, NICEATM will collaborate with ECVAM and the Japanese National Institute of Health Sciences to obtain additional data.

Nomination #4: Explore Ways of Alleviating Pain and Suffering for Animals Used in Ocular Irritancy Testing

Dr. Stokes said the process for this nomination will follow a similar path. This will involve a literature search for relevant methods; a Federal Register solicitation of appropriate information and data; contact industry and/or government experts; and preparation of a Background Review Document. The outcome of these activities will likely be organization of an expert workshop to review the available procedures and develop recommendations. NICEATM and ICCVAM are collaborating with ECVAM on their evaluation of the low volume eye test (LVET), which has been nominated to ECVAM for review.

Dr. Stokes concluded his presentation by mentioning a pending test method nomination from USDA on the *in vitro* Leptospira Potency test method. ICCVAM unanimously approved a request by the USDA representative to create an ICCVAM working group to provide comments on the proposed validation studies and coordinate eventual peer review. A status update on the nomination will be presented by Dr. Kulpa-Eddy as the next agenda item at this SACATM meeting.

Public Comment

Dr. John Gordon, Xenobiotic Detection Systems, updated SACATM on the validation progress for the LUMI-CELL recombinant bioassay for estrogenic endocrine disruptor compounds. This assay is one of the nominated endocrine methods. The assay was developed in collaboration

with Dr. Mike Dennison at the University of California-Davis. In brief, BG-1 cells are stably transfected with an estrogen-responsive luciferase gene in a reporter plasmid (pGudLuc7ere). Dr. Gordon said the resulting cell line is sensitive and responds to estrogenic chemicals in a time- and dose-dependent and chemical-specific matter. He presented several dose-response curves of organochlorine pesticides, pharmaceutical and steroidal estrogens, and clay borne products. Dr. Gordon said his company is just starting to characterize the antagonistic response and that tamoxifen is going to be used for the standard curve for antagonistic compounds. He felt that the assay fulfills many of the requirements presented by ICCVAM for a general screening program; however, funding for validation studies is difficult because endocrine disruptors are not yet regulated. The lack of validated studies makes regulation more difficult.

Dr. Sonnenschein asked why tamoxifen and not the ICI antagonist is being used as the full antagonist for the standard curve given that tamoxifen is not a full antagonist and is also a mild agonist. Dr. Clark responded that his company is just beginning to characterize the antagonist response and that he thought it is a good idea to include the ICI compounds as another control.

Dr. Sussman from Pfizer said the pharmaceutical industry has done a lot of work with *in vitro* endocrine disruptor systems, such as for evaluating occupationally relevant endpoints. He said a few poster and papers have been published on these systems. Dr. Stokes said he would follow-up and make sure they get all the posters and abstracts.

Discussion

Dr. Acosta asked two questions. First, he wanted to know what the overall budgets for NICEATM and ICCVAM are because he felt this information would help focus his questions since he is unsure of committee's role (e.g., is the role to provide feedback on funding of specific projects?). Second, Dr. Acosta wanted clarification on sources of funding once a project is approved; is it competitive and if so, can academic labs compete? Dr. Portier replied that NIEHS allocates about $2.5 million dollars a year for NICEATM and the ICCVAM process. Research that's funded by NIEHS goes through committees. If it's extramural, then it goes through the extramural funding committee for grants. Contract work goes through an internal agency review and then to the NIEHS council for review. Dr. Portier also noted that as the Director of the ETP, he had acted quickly on approving development of a background document on the estrogen and androgen receptor assays that were given high priority by SACATM at the last meeting. Dr. Portier emphasized that NIEHS is not the only agency on ICCVAM that can fund these activities. It is up to each agency to speak to how the do or do not fund the activities of ICCVAM. Dr. Portier said the role of SACATM is to provide scientific advice to all 15 agencies on ICCVAM. Dr. Curren noted that the nomination letter from EPA to ICCVAM-NICEATM included an offer to fund part of the activities. Dr. Merenda, EPA, confirmed this.

Dr. Stizel wanted clarification on whether the predicament outlined by Dr. Gordon is accurate: Is it true that no one would fund a validation study for endocrine disruption because EPA does not require those tests? Dr. Clark clarified the NIEHS had funded phase I and II SBIR studies, but the NIH does not really have a phase III process. Dr. Clark suggested that the SACATM make a recommendation that some process evolve to address this issue. Dr. Dean suggested thar the recommendation might be that the government agencies collaborate via ICCVAM to help resolve this issue. He understood it is not the role of NIEHS to fund translational studies, and he thought the agency that would use the test method on a regulatory basis should be the one involved in funding. Dr. Stitzel agreed that NIEHS should not be the only source of funding for the validation studies unless money is added to its budget for this purpose. She felt agencies

that are requesting these methods should bear funding responsibility; for the ED validation studies it would be EPA. Dr. Merenda said EPA does have an endocrine disruptor screening program funded for about $9 million in 2004. EPA has funded work through NICEATM for the initial estrogen and androgen receptor binding assays. EPA is now proceeding with validation studies for tissue-based assays given its statutory timeline to implement an endocrine program. EPA recognizes the merit of having *in vitro* assays, but resources are not being directed towards validation activity on methods that are further down the road. Dr. Stephens asked if available money is one of ICCVAM's prioritization criteria. Dr. Stokes replied that is it not, the criteria are science-based. Dr. Stokes said if ICCVAM, SACATM and the public all consider something to be a high priority, then that information will be considered by agencies that could potentially fund the activity.

Dr. Stephens made four points. First, although he was glad to see work on mechanistic assays that could prevent downstream animal use, he is concerned that these types of assays would be used to screen chemicals for additional animal testing. Second, he supported efforts to address the severe ocular toxicants first and he supported the emphasis on refinement (e.g., reducing pain and suffering). Third, he hoped the generation of new *in vivo* data by ICCVAM could be avoided by encouraging industry to come forward with existing data. Finally, he asked about the source for the rabbit eyes. Dr. Stokes replied that the rabbits are not euthanized solely for their eyes, but that they would be used for other procedures. Dr. Stitzel added that rabbit slaughter houses exist and certain companies get the eyes from the slaughter house.

Dr. Safe said it did not make sense to him that EPA is spending $9 million on endocrine disruption, but that it's not directed towards validating an assay. Dr. Merenda said EPA is mandated to establish a screening program using scientifically validated test methods. EPA's financial resources are directed towards validation of the range of Tier 1 screening and Tier 2 testing that has been recommended by Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC). He said Dr. Safe's question for EPA really addresses how many alternatives to pursue in parallel or which single assays should be pursued. EPA made the decision early in the process to fund tissue-based assays for estrogen and androgen receptor binding because in EPA's view those were more ready for application and EPA is already behind the statutory deadline for implementing the program. EPA is cognizant of the fact that many of the test methods it's validating would not be appropriate for large scale programs, but EPA is trying to get methods validated as quickly as possible so they can implement the program.

Dr. Goldberg asked Dr. Stokes and ICCVAM for clarification on follow-up of the literature review for ocular irritancy methods given that the extreme possibility exists are that either ICCVAM will conclude that no clear assay meets the objectives without additional work, or that one or two assays have enough published material to say that they are ready and have been validated by the literature and data that's available. Dr. Stokes replied that hopefully the outcome will be identification of one or more test methods with adequate validation data, and then ICCVAM can convene an expert peer review panel and make recommendations to the agencies. ICCVAM is interested in test methods that can detect both irreversible and reversible effects, but the first priority given current resources is to evaluate methods for irreversible effects. He expects that NICEATM staff, the ICCVAM working group and ECVAM staff will move this project quickly along. Dr. Goldberg said he is really concerned about eliminating tests that have a long history and continue to "almost make it" but ultimately fail validation. He wanted to know if there is a strategy for eliminating them from future consideration.

Dr. Portier clarified that there is nothing in the queue that is unfunded. Everything recommended by ICCVAM has been funded either by NIEHS, EPA or some other Federal agency.

Dr. Acosta reviewed the minutes from the previous meeting and noted that Dr. Portier had given a good description of funding by NIH and NIEHS, but Dr. Portier had indicated at the last meeting that more specific information could be provided on resources for individual components of alternative test method funding, such as the use of alternatives versus development versus validation. Dr. Acosta's key point was that it is still unclear what type of funding is available for a variety of validation studies. He also pointed out that in the August meeting Dr. Stokes said a survey would be sent to all the various agencies represented on ICCVAM requesting information on the use of animals and alternatives. Dr. Acosta expressed his frustration that information from these requests is not being reported back to SACATM. He said it is unclear how many dollars are available for each of these activities. Dr. Acosta asked for more information on support for alternatives by other agencies and whether he is to assume that the Xenobiotic Detection System proposal is being supported since Dr. Portier said that everything that came before his group is supported. Dr. Portier said the issue of SBIR funding is very important and he would present this to the director of NIEHS and suggest that it be brought before council because this is an issue for NIH as a whole. Ms. Amundson said this is an important point and presented several follow-up comments. She was also expecting a report or update from the other Federal agencies on their effort towards alternatives. She said that the endocrine budget for EPA is between $9 and $12.5 million since the Food Quality Protection Act passed. In addition, during fiscal years 2000 to 2001, animal protection agencies raised money specifically for research and development and validation under the High Production Volume (HPV) chemical agreement. It is her understanding that this money is being spent on "omics" research and not alternative test method development.

Dr. Dean summarized two main discussion points. First, the type of transcriptional assays proposed by Xenobiotic Detection Systems needs some level of support to be translated and validated. Agencies that would use such an assay represented within ICCVAM should bear some financial responsibility for supporting their validation. Second, those well-studied test methods that do not have utility should be eliminated from future consideration. Dr. Dean asked SACATM if they agreed with the priorities that Dr. Stokes presented. Dr. Stitzel felt that it is inappropriate for NICEATM to do all the background work on a test method that has already been through a validation effort several times. She suggested that there might be good summaries already available from prior validation efforts and that test method sponsors participate in background work. Dr. Stokes replied that previous validation efforts for *in vitro* ocular irritation did not go forward, in part, because they were evaluated as total replacements for both reversible and irreversible effects. The current evaluation will separate the evaluation of irreversible and reversible effects. The other major issue with prior validation efforts in this case is that the methods were not evaluated for their ability to predict current classification hazard categories. Dr. Stokes said that previous reviews would be considered. Dr. Stitzel commented that for the ocular methods, there has been a considerable amount of research by industry and it appears that industry is relying on NICEATM to organize and interpret the data. NICEATM.

Dr. Curren suggested in the future that topics with a lot of substance like the nomination presentation be presented in sections. He raised several questions about endocrine disruptor

activities. First, he wanted to know what is meant by the results of the peer-review committee on estrogen and androgen receptor assays being "largely accepted" by ICCVAM. Dr. Stokes replied that the recommendations were fully adopted. Dr. Curren asked for clarification on whether Xenobiotic Detection System had submitted an application for review by NICEATM or their methodology. Dr. Stokes replied the Xenobiotics had submitted a letter to NICEATM announcing its intention to nominate their method for validation studies. Xenobiotic Detection Systems was informed that additional information would need to be submitted before a recommendation on the priority could be made, such as pre-validation data, the study design, a list of proposed chemicals for validation studies and a standardized protocol. The package would be evaluated to see if it meets the minimum essential test components that were recommended by the expert panel. Dr. Curren thought the process is working. Dr. Stokes agreed and said a review of the proposed endocrine methods had not been completed; therefore, there is no recommendation for funding support at this time. Dr. Curren had several more comments on ocular irritation. First, he said that he may have a conflict because his company performs ocular irritation testing. He thought the effort had utility, but there have been calls in the past for additional data. He thought that there may be a review comparing these methods to the current GHS classification system. He suggested that SACATM might have a role in fundraising and identifying relevant data by working with trade organization connections.

XII. Update on the *In vitro* Vaccine Potency Tests for Veterinary *Leptospira* Vaccines

Dr. Kulpa-Eddy gave an abbreviated version of her presentation due to time constraints. She said the basic taxon of the *Leptospira* bacteria is the serovar and that it is differentiated by the antigens on it surface rather than how it looks under a microscope or its biochemical properties. Leptospires occur naturally in a wide variety of wild and domestic animals. In the natural host, the kidneys are colonized and the bacteria shed in the urine. Definitive (maintenance) natural host and specific serovars are the rat (icterohaemorrhagiae), raccoon (grippotyphosa), dog (canicola), cattle/swine (pomona), cattle/sheep (hardjo), and sheep/swine/hedgehog (bratislava). Humans are incidental host that can be infected by direct contact with infected urine or contact with infected urine via water and soil. Clinical manifestations are variable, depending on host (definitive or incidental), exposure dose, route of exposure, immune and hormone status of host, pathogenicity of the inocula, and previous exposure. Symptoms can range from inapparent infections to more serious effects. In the acute phase in incidental host, symptoms can be flu-like illnesses, hemolytic anemia, hemoglobinuria and jaundice. In the chronic phase in definitive host, symptoms can be kidney and liver damage, abortion and stillbirths (usually the first sign of herd infection). Immunity is generally humoral, life-long and serovar specific. Leptospirosis in animals is controlled by vaccine.

Hamster potency test (current)

The potency test is a hamster vaccine-challenge assay with three steps: 1) ten hamsters are vaccinated with a specified dilution of bacterin and ten others are held as controls; 2) all twenty hamsters are exposed to virulent challenge with appropriate serovar 14 days later; and 3) after 14 days, the numbers of live and dead animals are counted. A minimum of 80% of vaccinates must survive and a minimum of 80% of controls must die. There are several disadvantages of the test, a large number of animals are required; it is expensive, time consuming and labor intensive; and it exposes personnel to viable pathogenic organisms.

ELISA potency test (proposed)

Dr. Kulpa-Eddy discussed the proposed potency enzyme-linked immunosorbent assay (ELISA) test. The ELISA uses monoclonal antibodies prepared against a virulent culture. Dr. Kulpa-Eddy described that the bottom of each well is lined with capture antibodies (rabbit polyclonal). The test bacterin contains an unknown quantity of antigen, and it is compared to a reference bacterin containing a known quantity of antigen. A detection antibody is then added (mouse monoclonal), followed by a secondary antibody (goat anti-mouse immunoglobulin) attached to an enzyme. Addition of the substrate causes the enzyme to elicit a color change. If the antigen is not present, there is no color change. The advantages of the ELISA is that it measures a relevant antigen, there are no hamsters involved (and few cultures to maintain), it's less expensive ($640/hamster test versus $2/ELISA test), and personnel are not exposed to a human pathogen. There are several issues that need to be resolved before the ELISA test method can be taken forward in the validation process.

- Reference bacterin must be correlated to host efficacy (dogs, pigs, cattle). The USDA has a contract with Michigan State University to do this validation test (dogs first, then swine); however, this study is on-hold until a qualified challenge culture is available. A total of $750,000 is allotted for this test.
- Studies require qualified challenge cultures. The bacteria have been passed and maintained in hamsters for so long and have become so well adapted to hamsters that there is concern whether it can be used back in host animals. The host animals need to become sick to determine whether the vaccination is satisfactory. Currently the USDA is working on re-qualifying the challenge cultures.
- Limited supply of monoclonal antibodies. The mouse monoclonal antibodies were developed in the late 1980s or early 1990s. The USDA has since created bioreactor fluids in addition to the mouse monoclonals, but these have not been evaluated for equivalency.

Dr. Kulpa-Eddy outlined the strategy to address these issues. Media studies were completed in January 2004 and work is ongoing to qualify the pathogenicity of the challenge cultures (*Leptospira pomona* in swine has been done). Other activities include qualifying the bioreactor fluids to the monoclonal antibodies and assessing host animal passive protection. The latter effort is to ensure the specificity of the detecting antibody in the ELISA test. They also need to validate the reference bacterin in the hamster test, the ELISA test and back to the host animal.

Dr. Kulpa-Eddy said there are four Leptospires that have regulatory test requirements. The challenge culture qualification of *L. pomona* in swine has been completed and qualification in the dog is in process. Host animal passive protection testing is scheduled for September 2004; the reference bacterin validation is slated to begin August 2004 and will take approximately 18 months to complete. Timelines to complete reference bacterin validation for the other serovars are August 2004, October 2004 and December 2004.

Discussion

Dr. Willhite complemented Dr. Kulpa-Eddy on her presentation. Dr. Willhite pointed out from the August 2003 minutes that about 40,000 hamsters are used annually for Leptospira potency testing. That number, in combination with the total number of hamsters used in the United States reported in the USDA Animal Welfare Report (167,231), says that about 25% of all hamsters used are for the potency test. Dr. Willhite asked whether there are future plans to address the 120,000 mice used for *Erysipelothrix rhusiopathiae* and the 18,000 guinea pigs used for *Clostridials*. Dr. Kulpa-Eddy replied that the plan is to see how Leptospira proceeds and then explore these next. A monoclonal has been developed for *E. rhusiopathiae* and there

is the possibility of an ELISA test. She did not think alternatives are as far along for Clostridials and ELISA would not be the strategy used. Dr. Willhite suggested that SACATM should recommend a report from the FDA about human vaccine validations, specifically to find out if FDA is running studies through primates or some other species and how many animals are used. Dean said that is a reasonable request and he thought that most of them already use this type of potency assay, but that SACATM could ask FDA for their information. Dr. Dean said that these kinds of assays are obvious for determining antigen potency and he commended USDA for moving in this direction. Dr. Dean asked Dr. Kulpa-Eddy whether Leptospira modulates its antigenicity over time. Dr Kulpa-Eddy responded that she did not know, but could find out. Dr. Dean added that he understood that monoclonals were used in the original assay and that those could simply be propagated to produce more antibody for some type of commercial kit. Dr. Kulpa-Eddy said this is correct, but the mouse monoclonals were used in the pre-validation work to develop the ELISA test with and there is a very limited supply. USDA wants to qualify the bioreactor fluids for use in lieu of the mouse monoclonals. Dr. Portier wanted to understand how much of a reduction in animal use this assay would provide if the potency of the bacterium needed to be assessed every year in some animal species to make sure that it is actually potent before the efficacy is tested. Dr. Kulpa-Eddy said she thought, but was not positive, that the reference bacterin would need to be re-qualified every three years. Dr. Dean said he thought that if the original culture is cryo-preserved it wouldn't have to be re-qualified because the reason it changes is because it's been passed in the hamster and there is some sort of selection. Dr. Kulpa-Eddy responded that it's true the challenge culture would not have to be re-qualified, but the reference bacterin (the vaccine that's made to protect animals) would have to go back to the host animal from time to time to make sure it's potency has not decreased. Dr. Willhite commented that not only does this assay decrease test duration and cost, but it also reduce paperwork by decreasing reporting requirements. Dr. Stitzel ended the discussion by praising Dr. Kulpa-Eddy for a great presentation and progress on this issue.

XIII. Evaluation of the Under-Prediction Rate of *In Vivo* Dermal Corrosivity Test Methods

Part I: Introduction

Dr. Stokes acknowledged members of the ICCVAM Dermal Corrosivity and Irritation Working Group and other people engaged in this effort (Dr. Joe Haseman, NIEHS/NTP; Drs. Neepa Choski and Tice, ILS, Inc., NIECEATM). He also thanked several people and organizations for contributing data (Dr. Richard Hill, EPA; Marianne Lewis, EPA; Donnie Lowther, FDA; *In vitro* International Inc.; and the European Centre for Ecotoxicology and Toxicology of Chemicals)

The test method evaluated was the Draize rabbit skin test method. This method has been used since the 1940s although different versions have evolved along the way. The current version has been used since 1981 and involves applying a substance to intact skin with a patch for 3 minutes, 1-hour, and/or a 4-hour period. For Department of Transportation (DOT) hazard classification, a 3 minute and/or 1-hour exposure is required. Other agencies not interested in the DOT classification use the 4-hour period to assess corrosivity and irritation. At the end of the exposure period, the patch is removed and observations are made at 24, 48 and 72 hours. For irritation, erythema and edema are scored. For corrosivity, an observation is made for irreversible effects like eschar and necrosis. Animal welfare concerns prompted development and validation of *in vitro* test methods over the past 15 years. ICCVAM recently reviewed and recommended three types of *in vitro* assays: Corrositex, EpiDerm™/EPISKIN™, and the rat skin TER assay as screening assays in accordance with an internationally harmonized tiered testing

strategy adopted by the United Nations in 2003. This proposed use was recommended because 12 to 17 percent of the positive corrosive chemicals tested in the *in vitro* assays give false negative results. Because the effects of exposure to corrosive can lead to irreversible skin or eye injuries in humans, the *in vitro* assays were not recommended as complete replacements. According to the tiered testing strategy, a positive response in these assays can be classified as corrosive, but a negative response needs to be followed by further testing using appropriate test methods. Follow-up testing can included *in vitro* dermal irritation when those methods have been validated and accepted (there is an ongoing validation study of these methods). Animal testing is sequential and uses up to three rabbits. The first animal is tested and if a corrosive lesion develops, testing is stopped and the compound is classified as corrosive. If the response is negative, then a second animal is tested. Again, if a corrosive lesion is observed then testing stops, but if a negative response is noted in the second animal, a third animal is tested. If the third animal does not have a corrosive response, then the data from all three animals are used to meet the regulatory testing requirements for dermal irritation.

After the ICCVAM recommendations were published, ICCVAM received public comments stating that ICCVAM should recommend and agencies should adopt, these test methods as complete replacements for the rabbit assay. The comments were based on the assertion that the *in vitro* test methods are more accurate than the animal based methods. One comment cited the low interlaboratory reproducibility of the rabbit assay presented in a study published by Weil and Scala in 1971. Other claims were made that the rabbit assay has a 20-25% false negative/false positive rates for corrosivity (no data were provided to substantiate this claim).

Dr. Stokes said the Weil and Scala study evaluated the reproducibility of the Draize *in vivo* rabbit skin test method within and among 24 laboratories for 10 substances. This study is the only formal evaluation of the reproducibility of the Draize *in vivo* rabbit skin test, although the analysis was really for irritation and not corrosivity. The three main conclusions from the study were:

1. Moderate intra-laboratory reproducibility
2. Low inter-laboratory reproducibility
3. Primary reasons for the low inter-laboratory reproducibility were attributed to the subjective nature of the visual observations and variations in procedures among labs

Dr. Stokes explained that the Weil and Scala study also had two main limitations. First, the standard protocol used was different from the Draize *in vivo* rabbit test method protocol in use since 1981. For example, the Weil and Scala studies used a 24-hour exposure period versus the current maximum of 4-hours exposure, and this prolonged exposure actually resulted in corrosive lesions by some of the chemicals. Also, GLP guidelines had not been established in 1971; the impact of this is not known. The objective of the NICEATM study is to evaluate the likelihood of under-predicting a corrosive substance as a non-corrosive in the current rabbit dermal corrosivity test. The data may assist in establishing an acceptable false-negative rate for corrosive effects for *in vitro* test methods proposed as complete replacements for the rabbit skin test. A complete replacement is a test where no *in vivo* confirmation would be performed. NICEATM gathered data from UN packing groups on corrosive severity. There are three UN packing groups: Packing Group 1 (corrosive burn within 1 hour after a 3 minute exposure); Packing Group 2 (corrosive burns within 14 days after a 1-hour exposure); and Packing Group 3 (corrosive burns within 14 days after a 4-hour exposure). Overall, only limited packing data are available. NICEATM also tried to identify corrosivity data for humans, but none were found so it was determined that it is not possible to assess the false negative and/or false positive rates of

the rabbit dermal corrosivity test method for humans. Dr. Stokes said there are no reports of human corrosive burns following exposure to non-corrosive substances.

NICEATM compiled an *in vivo* corrosivity database by requesting data from Federal agencies and other sources via a Federal Register notice. The data come from corrosivity studies using the current exposure for the rabbit skin test protocols recommended by US Federal Agencies (e.g., FDA, EPA, CPSC, DOT) and the OECD (Test Guideline 404). The current database has 171 substances from 185 separate studies. Data sources were: *In Vitro* International (Bio-Technics), ECETOC, EPA (OPPTS), and FDA (CFSAN). Some of the data is for substances that were unidentified commercial products with unknown formulations and chemical composition. Most substances were only tested in one study. Nine chemicals were tested in 2 studies and one chemical each was tested in 3 and 4 studies. The numbers of animals used per study ranged from 1 to 6. Dr. Stokes explained that it was standard until the mid-1990s to use 6 animals. The current maximum number of animals is three. Many studies only used one animal because the first animal produced a corrosive lesion and no further testing was required. A limitation of the database is that potency subcatagories are not known for most chemicals, only corrosive versus non-corrosive. This evaluation is only preliminary and NICEATM continues to seek high quality data to add to the database. Currently, NICEATM is collaborating with EPA OPPTS to obtain microfiche reports for 2400 commercially available chemicals with dermal test results from the EPA TSCATS database. However, the availability of individual animal data and distribution of corrosives chemicals are unknown.

Part II: Data Analysis

Dr. Joe Haseman, retired from NIEHS, presented the analysis of the database. He began by defining the false negative rate of a corrosivity test is the probability that a corrosive substance will not produce a positive response when subjected to the test (i.e., will produce a response that "falsely" identifies the substance as non-corrosive in the rabbit model). He said the false negative rate depends upon two factors, 1) the specific corrosivity test used, and 2) the responsiveness of the animals to the corrosivity test (mean response rate and variability in response). Dr. Haseman's analysis was based on two assumptions. First, that corrosivity is based on a three animal test, in which one or more positive responses indicates that a test substance is corrosive. The second, is that the distribution of test substances in the database, in terms of corrosivity (i.e., the proportion of responding rabbits), is representative of the "real world" of corrosive substances. Dr. Haseman said the first assumption is relatively straightforward, but the second is the "soft" part of the false negative calculation. To address the second assumption, Dr. Haseman said the strategy was to calculate nine false negative rates, each one based on slightly different assumptions about the distribution of the response of chemicals in the database and consistent with the sample data. Dr. Haseman explained that he calculated the estimated range false negative rates by filling in the cells of the following three by three table:

Basis of False Negative Analysis

□	**Approach 1: Based on Studies**	**Approach 1: Based on Test Substances**	**Approach 2: Average Response Rate**
All Data Used	□	□	□
1 Animal Tested Excluded	□	□	□

1 and 2 Animal Tested Excluded	□	□	□

Approach 1
For Approach 1, Dr. Haseman used a specific distribution of response rate that mimics the data in the database. Approach 1 could be either based on the number of studies (n=185) or the number of test substances (n = 171). In the later case, responses for the same test substance from multiple tests were averaged. Dr. Haseman noted that 11 chemicals were multiply tested and the results appeared to be very reproducible.

Approach 2
For the second approach, Dr. Haseman assumed there is an average response. Over all the studies and over all the chemicals, he estimated the average likelihood that an animal would be responsive and then assumed that this response rate applied to all the chemicals in the database. He believes this represents a best case scenario and that the false negative rates in this column would be underestimates.

Data Used
Dr. Haseman explained what the rows meant in the table presented above. In addition to using all the data, he used scenarios based on removing the one and two animal studies. He said a case could be made that since 56 of the studies involved a single-animal test (it was positive, so testing was stopped), the response rate for those 56 studies would be 100% and this might skew the distribution towards responsiveness. But, if more animals had been tested, then one or two might have been unresponsive. For the same reason, the studies with two animals were removed in the third row (only test with three or more animals used). Dr. Haseman thought the last row is probably producing slight overestimates of false negative rates. He felt that the most representative false negative rates would be from the following cells: approach 1 (studies) – all data; approach 1 (test substances) – all data; approach 1 (studies) – 1 animal test excluded; and approach 1 (test substances) – 1 animal test excluded.

Analysis
Dr. Haseman then presented the distribution of substances in the database for approach 1. He calculated a range of positive response rates (16.7 to 100%) based on six possible approach I scenarios: 1) based on studies, all data, 2) based on studies, 1 animal tested excluded, 3) based on studies, 1 and 2 animal tested excluded, 4) based on test substances, all data, 5) based on test substances, 1 animal tested excluded, and 6) based on test substances, 1 and 2 animal tested excluded. He found that well over half the compounds produce positive responses in 100 percent of animals; these chemicals are not of concern for false negative outcomes. The problematic chemicals in terms of false negative rates are the small number that produced a response in one out of three, two of six or one of six animals.

Dr. Haseman explained how he calculated a false negative rate, defined as being one that, in three successive tests, gave a negative response. The simple way to calculate the false negative rate for each probability is to take 1 minus that rate and raise that number to the third power (i.e., the likelihood of getting a negative once, twice, or three times). So, if an animal had a 50 percent chance of responding then the chance that three animals wouldn't respond would by _ x _ x _ = 1/8 or 12.5 percent. Dr. Haseman emphasized that there won't be a false negative if probability of positive response is 100%, but when this probability decreases the

concern is higher. The worst case in his table is if the underlying likelihood of a randomly selected animal for a given chemical is only 1 in 6 that it will respond, chances are little better than 50/50 that the chemical in a three-animal assay would be non-corrosive. He also said that the negative response likelihood calculations were inconsistent with the speculated false negative rate of 20 to 25 percent. This would require an overall response rate around 30 to 40 percent, but the observed overall response rate was between 70 to 80 percent.

Dr. Haseman presented an example of a false negative calculation using approach 1, all data. The overall estimated false negative rate for this scenario was 4.9%. A probability of positive response of 100% did not contribute to the false negative rate whereas a probability of positive response of 16.7% contributed the most.

Dr. Haseman than presented Approach 2, the distribution of animals with a corrosive response in the database based on totaling how many animals responded over the total at risk. When all the data were used, this number was 78%. When 1 animal tested studies were excluded, the number was 75.4%. The proportion of animals with a corrosive response was 71.1% when 1 and 2 animals tested studies were excluded. He presented a sample false negative rate based on all available data: positive response rate = 412/528 or 0.78. The false negative rate calculation is: $(1\text{-}0.780)^3 = (0.22)^3 = 0.0106$ or 1.1%.

Dr. Haseman presented the range of estimated false negatives;

Estimated False Negative Analysis

□	**Approach 1: Based on Studies**	**Approach 1: Based on Test Substances**	**Approach 2: Average Response Rate**
All Data Used	4.9%	5.0%	1.1%
1 Animal Tested Excluded	7.1%	7.1%	1.5%
1 and 2 Animal Tested Excluded	10.3%	9.2%	2.4%

He reminded SACATM that these data are preliminary and that additional data are being sought by NICEATM. He felt the false negatives based on Approach 2 were probably a little low and that those in the last row (1 and 2 animals tested excluded) were a little high, because about half of the most responsive chemicals were removed from the analysis, skewing the database towards non-responsive. He felt the most reasonable estimate would be between 5 and 7 percent. Dr. Haseman concluded with three points:

- Within the limits of assumptions, the false negative rates ranged from 1.1% to 10.3%
- The false negative rate most likely to be representative of this group of corrosive substances is from 5 to 7%
- Additional data will allow for refinement of these false negative rates, but it unlikely to significantly change these estimates.

Public Comment

There were no public comments.

Discussion

Dr. Curren felt this subject, determining quantitative criteria for accepting or rejecting of an *in vitro* test, was the most important presentation of the day. He applauded ICCVAM for being more quantitative in what is meant by "criteria for validation of a test." He was concerned that the false negative number presented only reflected intra-laboratory variability and did not incorporate differences between labs. Dr. Haseman said that to his knowledge all the numbers, even those multiply tested, were done in a single lab. Dr. Curren said the agencies would really be interested in inter-laboratory variability because they receive data from a number of different laboratories. Dr. Curren said he is not criticizing the analysis because it sounded like the data weren't available for that analysis. He noted that the Weil and Scala analysis did include data where at least several compounds were tested in multiple labs. In this study, there were vast differences between labs. For example, one classified eight animals as corrosive and another had zero animals as corrosive. Dr. Curren suggested that SACATM, either in conjunction with ICCVAM and others, have a small focused workshop on developing quantitative information to determine whether an *in vitro* test is as good as an *in vivo* test. Dr. Hayes asked for clarification on whether all the tests had been done in a single laboratory. Dr. Haseman clarified that the data were generated in different laboratories, but that no single chemical was tested in multiple laboratories. He said the data reflect the average intra-laboratory variation.

Dr. Monteiro-Riviere, a lead discussant, asked whether the class of compound or the volatility of a compound was considered and how would this affect the analysis. Dr. Haseman said this information was not considered, because he did not have that information. This sort of calculation could be conducted for any given class of chemical if the distribution of responsiveness within the class could be provided. He added that although the class could affect the outcome, the overall rate of responsiveness would still need to be 30 to 40 percent to yield false negative rates in the 20 to 25% range.

Dr. Green recalled that NICEATM conducted an expedited review of the *in vitro* test methods because the three assays had been validated by ECVAM. He asked Dr. Stokes whether there is a systematic manner in which NICEATM/ICCVAM evaluate whether a test method is appropriate for expedited review. Dr. Stokes said that ICCVAM implemented an expedited review process to look at methods that have been carefully reviewed by other organizations. If ICCVAM agrees with the interpretation of the validation study, then ICCVAM makes draft recommendations on the test methods and publishes these for public comment. If there is no significant disagreement from the public or SACATM, then ICCMAM will finalize the recommendations and send them forward for consideration by U.S. agencies. For the *in vitro* dermal studies, ICCVAM agreed that the review was complete. Dr. Stokes said he could discuss the expedited review process in more detail at the next SACATM.

Dr. Flournoy agreed that this was a very important presentation and one that raises a lot of questions regarding quantification. She had several questions and comments. She wondered if the negative rates based on testing three animals in a group could be used to predict what the false negative rate would be using a sequential testing procedure. She felt it important that background prediction rates provide a target for alternative methods to achieve. She also felt that it would be important to know how the prediction targets for different subclassification might change since they could alter the target that alternatives are trying to achieve. Also, different targets for different subclassification of chemicals may present multiple testing problems that would need to be accounted for. Dr. Flournoy thought that testing continued until a positive response occurred and she suggested a sequential approach. Dr. Haseman replied that the

false negative rates apply for a sequential test that stops at three because the calculations do not depend on whether the animals were tested sequentially or all at one. The only way to get a false negative is to test three non-responders. The advantage to sequential testing is that you can stop if there is a positive response, but the false negative estimates won't change.

Dr. Haseman thought it would be worthwhile to subgroup the 171 chemicals by class to see if there were some commonality to the chemicals with low responder rates (16 and 33%). Dr. Dean said it was a good recommendation and asked if the committee agreed with that. Dr. Portier commented that this type of test could not be done independent of an *in vitro* assay and the important question for ICCVAM is what information this test provides above and beyond the *in vitro* assay. He said the highly corrosives would likely not be tested in animals because they would be positive in the *in vitro* assay. Therefore, the false negative rate may be higher because the *in vivo* test would be used only after a negative *in vitro* study, so the *in vivo* test may be confronted with compounds with more variable response. He suggested that it would be worthwhile to get data on the *in vitro* assays for the same compounds, do a screening and provide an update on type I error that reflects how much of a change in type I error you get by adding one, two, or three animals in a follow-up study. Dr. Haseman said this is an interesting idea and that the more strongly the *in vivo* and *in vitro* data are correlated, the worse the type 2 error of the follow-up would be in the *in vivo* test because it would only be used for the weak corrosives. Dr. Dean said this would be a good way to link the two assays and asked if the committee agreed with that recommendation. Dr. Haseman asked NICEATM staff how much *in vitro* data were available for the 171 chemicals. Dr. Tice said that virtually all the chemicals from Corrositex have corresponding data, but for the chemicals that came from FDA and EPA the answer is most likely none at all. Dr. Curren clarified that the InVitro International Inc. chemicals were used in the validation study, so there would be more than just Corrositex available. Dr. Haseman said his concern is that if the correlation is very good, then there may only be a few chemicals that were *in vitro* negative and *in vivo* positive. Dr. Smith encouraged seeking additional data and asked whether the EPA TSCATS database would coincide with the a similar database managed by the OECD SERTS database. She felt more *in vivo* studies should not be conducted to develop additional data on corrosivity.

Dr. Stephens said it is important not to forget the discussion last time this topic came up at SACATM with respect to how this issue was reviewed by OECD. The Europeans were satisfied with the combination of the *in vitro* test with some physical measurements, like pH, that essentially eliminated the false negative rate. Dr. Curren suggested holding a small workshop on discussing the analysis of the data because there are dissenting opinions as to the analysis of the data and conclusions about what under-prediction rates are acceptable. Dr. Flournoy commented that it is important to be clear about which level of error rates are being discussed, either the individual test or the decision to go forward with a test measurement procedure.

Dr. Tice assured the panel that NICEATM and ECVAM are jointly evaluating the performance characteristics of the *in vivo* irritation assay and collaborating on efforts to validate *in vitro* dermal irritation test methods. ECVAM conducted its own analysis of dermal irritation using the same data used by Dr. Haseman, with some additional data they located, and different statistical approaches and arrived at an under prediction rate within a few percentage points of the under prediction rates presented by Dr. Haseman. He added that one of the items under discussion with ECVAM is a potential joint workshop addressing statistical approaches for evaluating *in vivo* data. Without this type of analysis, it is difficult to evaluate whether an *in vitro* test method can be used as a partial or full replacement. Dr. Dean asked the committee if they

endorsed the idea of holding a workshop around the issue of statistical analysis of *in vivo* prediction rates and how to apply this information to *in vitro* assays. He acknowledged agreement from the committee.

Dr. Portier asked if the committee members were interested in seeing an analysis that compares the European and ICCVAM approaches. *In vitro* studies could be conducted for the chemicals that have been tested *in vivo*. The *in vitro* data could then be analyzed in combination with information on pH or structure to see if the chemical is correctly categorized based on the *in vivo* findings (the European approach). The ICCVAM approach of using animal data to follow-up on negative *in vitro* findings could also be analyzed since the animal data already exists. Dr. Stitzel said this is exactly the type of analysis that needs to be done, but there should be some indication from ICCVAM how the results would be used. Dr. Stokes commented on the idea of reducing the false negative rate to zero by incorporating informations such as pH. ECVAM staff have published a paper that used pH in conjunction with information on structure activity relationship (SAR). However, the SAR model used has not been validated, and the pH methodology has not been standardized and used for all of the existing chemicals in the validation database. Dr. Stokes said the false positive rate using this tiered decision strategy is about 25%, so it's important to strike a balance between the false negative and false positive rates. Dr. Merenda said ICCVAM should discuss the issue, but he thought the type of analysis Dr. Portier described would be useful. Dr. Curren said although he still approves of the expedited review process, it is important that an expedited review does not take away from the open peer-review process that distinguishes U.S. and European review.

XIV. Working Group Reports

Strategic Planning Working Group (Chair: Dr. Stitzel; Rapporteur: Dr. Curren)
Dr. Stitzel summarized the outcome of the Strategic Priorities Working Group. The group discussed three topics: the ICCVAM Strategic Plan, validation of discret mechanistic tests, and strategies to make SACATM operate more effectively.

1. *ICCVAM Strategic Plan*
   - There should be interaction between the NTP Vision for the 21st Century and the ICCVAM Strategic Plan. Dr. Stitzel said neither mentioned each other and there should be additional consideration as to how they intersect.
   - Since other groups (e.g., ISLI, EPA) have toxicological research programs addressing the use of new technologies, ICCVAM should act as a conduit for transferring information about methodology appropriate to the legislative mandate of each of the ICCVAM agencies.
   - ICCVAM could assist NTP with its goals (e.g., giving guidance on translating newly developed methods into assays useful to the regulatory agencies) and NTP could help with ICCVAM's needs (e.g., developing test methods that supply toxicological information needed by a specific agency)
   - Both the NTP Vision and the ICCVAM Strategic Plans documents could use more forward-thinking language.
   - Other suggestions or comments should be transmitted by the full SACATM to ICCVAM

2. *Validation of discrete mechanistic test (e.g. validations that go beyond a single test replacing a single test)*
    - There has been considerable discussion about this problem in the past, but questions still remain. We suggest a working group of SACATM, ICCVAM and ECVAM be tasked with creating a white paper that carefully defines the question and suggests a way forward (using examples).

3. *How can SACATM operate most effectively?*
    - SACATM should have active participation in setting the agenda (suggestion of the Chair and representatives from each working group)
    - Assume that much of the meeting documentation has been read by the members, so that presentations (especially update presentations) can be shortened to provide more time for discussion.
    - Encourage the lead discussants for each discussion topic to confer beforehand to agree on the most cogent comments and assure that comments aren't repetitive. However, all SACATM members need to participate in the actual discussions.
    - SACATM member(s) should have the opportunity to sit as observers at ICCVAM meetings and select advisory committee meetings that deal with issues relating to alternative methods.
    - Working groups could review NIEHS peer-reviewed and funded SBIR alternative grants and contracts to help identify priority methods which may have near term applications.
    - Working groups could also look at information specific to ICCVAM priorities that are beyond ICCVAM's current resources.
    - Send out background materials 3 to 4 weeks in advance.
    - Work on a process to formalize the outcome of a SACATM discussion, perhaps in the form of a vote or recommendation.
    - Develop working groups of SACATM to investigate specific areas that are of importance to ICCVAM, NICEATM or SACATM committee as a whole, i.e., to provide more direct assistance to ICCVAM. An example might be reviewing NIEHS peer-reviewed and funded SBIR alternatives grants and contracts to help identify promising methods that might have near term application. Another example might be looking at information specific to ICCVAM priorities that are beyond their current resources.

Dr. Portier thanked Dr. Stitzel for her suggestion and said that FACA subcommittees generally need to have a task, so that task would need defining before proceeding. He acknowledged the recommendation to have background materials four weeks in advance. Dr. Portier said that the agenda is already discussed with the SACATM chair (Dr. Dean) and he would have no problem extending this to a larger group of SACATM members. However, he said he is responsible for presenting topics that the agencies are asking to be raised. Dr. Stitzel emphasized that she is not saying that the presentations are of poor quality, but that the presentation time for updates could be decreased since the materials are provided in the background material packed. Dr. Stitzel said she would rather have more discussion time. Dr. Dean suggested that the discussants work together on a unified presentation that includes a recommendation. Dr. Willhite said if this is what happens the materials need to be distributed well in advance of the meeting so that there is time to delegate assignments. Dr. Portier reminded the committee that this is not a consensus committee and the idea of working together in advance to develop a consensus is not expected. So, the lead discussants can work together in advance, but SACATM should provide individual input. However, if there is a consensus, that should be presented. Dr. Stokes said it is a good suggestion for ICCVAM (through the ICCVAM chair and

NICEATM director) to have input from the SACATM Chair and other SACATM representatives. Dr. Flournoy said the data analysis example is a good one to illustrate the need of getting information out earlier because this would have allowed for more time to consider the material and also to raise points of clarification. Dr. Dean agreed with Dr. Flourony and clarified that he didn't mean to imply that SACATM should try to get consensus, but that there should be some uniformity in how this material is presented to the group. Dr. Portier cautioned that there are legal issues dictating how committees like SACATM work that it becomes problematic for committee members and presenters to correspond in advance and make a case for a particular opinion. He added that SACATM is an advisory committee and they should be commenting on the case presented to them, and if that case is not complete, then that should be reflected in the committee's advice.

Test Method Needs and Evaluation/Validation Priorities Working Group (Chair: Dr. Green; Rapporteur: Dr. Hayes)
Dr. Hayes said that the working group spent a considerable amount of time talking, and felt there are some priorities that could be identified. The major points raised in the working group were:

- Support state of the science evaluations of priority areas for alternative test method development/nomination/validation. NICEATM should be actively looking for those methods that are strongly scientifically based that the agencies need.
- The working group agreed that acute eye irritation and corrosivity, dermal irritation and acute systemic toxicity are priorities. Also, the USDA vaccine program should be at the top of the list, since it could show a huge reduction in animal use in a relatively short period of time. Endocrine disruption is also a priority.
- The working group felt the there should be a more proactive effort to look at new technologies, such as those in the genomic area that could be useful to various agencies. Dr. Hayes included the concept of biomarkers as something that could be potentially useful.
- The working group thought there were two pathways that methods could follow based on the availability of test methods. First, if promising methods are available, then these should move into the validation process to become validated methods. Second, if there are no promising methods, or more potential methods need to be found, then funding test method development would be appropriate (e.g., Request For Applications)
- Provide financial support to maintain interactions (via SACATM or ICCVAM members, or NICEATM staff) with ECVAM.

Dr. Portier commented that it is very unlikely that he would fund another Federal agency to send a Federal employee to a meeting that the other agency feels is important. He said that NIEHS submitted an official invitation to ECVAM to designate an ad hoc member to the SACATM. He reminded SACATM that ICCVAM and ECVAM are two separate entities and it could be difficult to think about a strategic alliance between the two. Dr. Hayes said the point is to minimize duplication of effort. Dr. Portier responded that is why an ECVAM representative is invited to the SACATM meetings. Dr. Hayes said that was why a NICEATM representative should be sent to the ECVAM meetings. Dr. Portier said he would if asked by ECVAM and funds were available. He also said it would be better if the representative were put on an advisory committee so they could see the overall program rather than pieces. Dr. Portier added that he had yet to send somebody to an invited ECVAM meeting, but that he didn't want to impose on ECVAM unless invited. Dr. Schechtman said it is important for the committee to realize that

ECVAM has repeatedly invited ICCVAM and NICEATM members to its various meetings and has conferred official Observer status to ICCVAM-NICEATM on the ECVAM Scientific Advisory Committee (ESAC); Dr. Stokes, as NICEATM Director, and he as ICCVAM Chair regularly attend the these meetings. The invitations include those for participating in the ESAC meetings, ECVAM workshops, working group meetings, task force meetings, and more. Dr. Stitzel suggested that working groups could perhaps develop white papers on topics. Dr. Portier responded that it would not be the job of SACATM to write a white paper, but rather it would be more appropriate for SACATM to advise on the correct experts to draft it and review it and see if the paper were complete. Similarly, it would not be the job of an advisory committee to conduct a workshop, but rather advise ICCVAM-NICEATM of the need to hold one.

Dr. Dean adjourned the meeting at 12:36 p.m.

I. Background

FDA, with input from an ad hoc workshop and an advisory committee, first issued guidance on osteoporosis drug development in 1979. The guidance was issued in response to the need for effective and safe drugs to prevent and treat osteoporosis. The agency revised the guidance in 1984. Most recently, FDA issued the 1994 draft guidance entitled ''Guidelines for Preclinical and Clinical Evaluation of Agents Used in the Prevention or Treatment of Postmenopausal Osteoporosis.''

The 1994 draft guidance recommends study designs, patient populations for study, and techniques for evaluating skeletal mass and fracture frequency that are considered central to demonstrating the efficacy and safety of drugs used to treat and prevent osteoporosis. Since issuance of the 1994 guidance, a number of drugs have been approved for the prevention and treatment of osteoporosis. In general, approval of these drugs was based on favorable bone mineral density and decreased fracture incidence from 2- and 3–year placebo-controlled trials.

Results from these trials and other published data have raised a number of issues and questions that the agency plans to address in an updated draft osteoporosis guidance. To aid in the development of the draft guidance, FDA is requesting comment on the 1994 draft guidance. The agency seeks specific comment on the following questions:

∞Is it appropriate to continue to use placebo controls in fracture end-point trials?

∞Do fracture end-point trials need to be 3 years in duration, or could shorter studies provide adequate evidence of a new osteoporosis drug's effectiveness and safety?

The 1994 draft guidance was issued before the 1997 publication of FDA's good guidance practices (GGPs) regulation (21 CFR 10.115). In accordance with the GGPs, the agency will take into account any comments received on the 1994 draft guidance, develop a new draft guidance, and make it available for comment. When finalized, that guidance will represent the agency's current thinking on the preclinical and clinical evaluation of agents used in the prevention or treatment of postmenopausal osteoporosis. Agency guidance does not create or confer any rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statutes and regulations.

II. Comments

Interested persons may submit to the Division of Dockets Management (see **ADDRESSES**) written or electronic comments on the 1994 draft guidance. Two copies of mailed comments are to be submitted, except that individuals may submit one copy. Comments are to be identified with the docket number found in brackets in the heading of this document. The 1994 draft guidance and received comments are available for public examination in the Division of Dockets Management between 9 a.m. and 4 p.m., Monday through Friday.

III. Electronic Access

Persons with access to the Internet may obtain the document at either *http://www.fda.gov/cder/guidance/index.htm* or *http://www.fda.gov/ohrms/dockets/default.htm*.

Dated: January 30, 2004.

William K. Hubbard,

Associate Commissioner for Policy and Planning.

[FR Doc. 04–2999 Filed 2–10–04; 8:45 am]

BILLING CODE 4160–01–S

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

National Institute of Environmental Health Sciences; Notice of a Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods

Pursuant to section 10(a) of the Federal Advisory Committee Act, as amended (5 U.S.C. Appendix 2), notice is hereby given of a meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) on March 10–11, 2004, in the Hyatt Regency Hotel, One Bethesda Metro Center, Bethesda, MD (301–657–1234 or 800–233–1234). The meeting begins each day at 8:30 a.m. The SACATM provides advise on the statutorily mandated duties of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the activities of the NTP Center for the Evaluation of Alternative Toxicological Methods (NICEATM).

Agenda

The meeting is being held on March 10–11, 2004 from 8:30 a.m. until adjournment and is open to the public with attendance limited only by the space available. Individuals who plan to attend are asked to register with the NTP Executive Secretary (Dr. Kristina Thayer at the NTP Liaison and Scientific Review Office, NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709; telephone: 919–541–5021; facsimile: 919–541–0295; or E-mail: *thayer.niehs.nih.gov).*

Persons needing special assistance, such as sign language interpretation or other reasonable accommodation in order to attend, are asked to notify the NTP Executive Secretary at least seven business days in advance of the meeting (see contact information above).

A preliminary agenda is provided below. A copy of the agenda, committee roster, and any additional information, when available, will be posted on the NTP Web site (*http://ntp-server.niehs.nih.gov*) under ''What's New'' or available upon request to the NTP Executive Secretary (contact information provided above). Additional information about SACATM is available through the NICEATM/ICCVAM Web site (*http://iccvam.niehs.nih.gov*) under ''Advisory Committee''. Following the meeting, summary minutes will be prepared and available at this Web site and upon request to the NTP Liaison and Scientific Review Office (contact information above). Information about NICEATM and ICCVAM activities can also be found at the NICEATM/ICCVAM Web site (*http://iccvam.niehs.nih.gov*) or by contacting the Director of NICEATM, Dr. William Stokes (919–541–2384, or e-mail: *niceatm@niehs.nih.gov).*

Preliminary Agenda

Scientific Advisory Committee on Alternative Toxicological Methods—March 10–11, 2004

Hyatt Regency Hotel, 301–657–1234 or 800–233–1234, One Bethesda Metro Center, Bethesda, MD 20814.

March 10, 2004

8:30 a.m.

1. Call to Order and Introductions
2. Welcome and Remarks from NIEHS/NTP
3. Welcome and Remarks from ICCVAM Chair
4. Update on Activities of the NTP Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)
5. Update on Activities of the European Centre for the Validation of Alternative Methods (ECVAM)
6. Toxicology in the 21st Century: The Role of the National Toxicology Program

a. Public Comment

7. Update on Animal Use

12 p.m.
Lunch Break (on your own)
1 p.m.
8. ICCVAM Strategic Planning Process
a. Public Comment
9. ICCVAM Recommended Performance Standards for In Vitro Dermal Corrosivity Methods
a. Public Comment
10. Evaluation of the Predictivity of In Vivo Dermal Corrosivity Test Methods
a. Public Comment
11. Overview of ILSI/HESI Subcommittee's Activities on Identification of Biomarkers of Toxicity and Summary of First Meeting
12. Validation of Genetically Modified Mouse Models
a. Public Comment
5 p.m.
Adjourn

March 11, 2004

8:30 a.m.
1. Introductions and Call to Order
2. ICCVAM–NICEATM–ECVAM Workshop on Validation of Toxicogenomics-Based Test Systems
a. Public Comment
3. In Progress Test Method Evaluation Nomination: In Vitro Test Methods for Identifying Substances Causing Irreversible Ocular Damage
4. New Test Method Nominations: EPA Test Method Nomination for Test Methods to Identify Negative, Mild, and Moderate Ocular Irritants (*i.e.* Those With Reversible or No Effect)
a. Public Comment
11:30 p.m.
Lunch (on your own)
12:30 p.m.
New Test Method Nominations continued: In Vitro Vaccine Potency Tests for Veterinary Leptospira Vaccines
a. Public Comment
6. Report on the ECVAM Workshop on In Vitro Replacements for Acute Systemic Toxicity
a. Public Comment
2:45 p.m.
7. Other Issues
3:15 p.m.
Adjourn

Public Comment Welcome

Public input at this meeting is invited and time is set aside for the presentation of public comments on any agenda topic. Each organization is allowed one time slot per agenda topic. At least 7 minutes will be allotted to each speaker, and if time permits, may be extended to 10 minutes. In order to facilitate planning for this meeting, persons wishing to make an oral presentation are asked to notify the NTP Executive Secretary (contact information above) by March 1, 2004, and to provide their name, affiliation, mailing address, phone, fax, e-mail, and sponsoring organization (if any). Registration for oral comments will also be available on-site, although time allowed for presentation by on-site registrants may be less than that for pre-registered speakers and will be determined by the number of persons who register at the meeting.

Persons registering to make oral comments are asked, if possible, to provide a copy of their statement to the NTP Executive Secretary (contact information above) by March 1, 2004, to enable review by the SACATM and NIEHS/NTP staff prior to the meeting. Written statements can supplement and may expand the oral presentation. If registering on-site and reading from written text, please bring 40 copies of the statement for distribution to the SACATM and NIEHS/NTP staff and to supplement the record. Written comments received in response to this notice will be posted on the NTP Web site (*http://ntp-server.niehs.nih.gov*) under ''What's New''.

Persons may also submit written comments in lieu of making oral comments. Written comments should be sent to the NTP Executive Secretary and should be received by March 1, 2004, to enable review by the SACATM and NIEHS/NIH prior to the meeting. Persons submitting written comments should include their name, affiliation, mailing address, phone, fax, e-mail, and sponsoring organization (if any) with the document.

Background

The SACATM was established January 9, 2002 to fulfill section 3(d) of Public Law 106–545, the ICCVAM Authorization Act of 2000 [42 U.S.C. 285l–3(d)] and is composed of scientists from the public and private sectors (**Federal Register:** March 13, 2002: Vol. 67, No. 49, page 11358). The SACATM provides advice to the Director of the National Institute of Environmental Health Sciences (NIEHS), the Interagency Coordinating Committee on the Validation of Alternative Toxicological Methods (ICCVAM), and the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) regarding statutorily mandated duties of the ICCVAM and activities of the NICEATM. The committee's charter is posted on the Web at *http://iccvam.niehs.nih.gov* under ''Advisory Committee'' and is available in hard copy upon request from the NTP Executive Secretary (contact information above).

Dated: February 2, 2004.

Samuel Wilson,

Deputy Director, National Institute of Environmental Health Sciences.

[FR Doc. 04–2931 Filed 2–10–04; 8:45 am]

BILLING CODE 4140–01–P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

National Cancer Institute; Notice of Meeting

Pursuant to section 10(a) of the Federal Advisory Committee Act, as amended (5 U.S.C. Appendix 2), notice is hereby given of a meeting of the Advisory Committee to the Director, National Cancer Institute.

The meeting will be open to the public, with attendance limited to space available. Individuals who plan to attend and need special assistance, such as sign language interpretation or other reasonable accommodations, should notify the Contact Person listed below in advance of the meeting.

Name of Committee: Advisory Committee to the Director, National Cancer Institute.

Date: March 2, 2004.

Time: 2 p.m. to 4 p.m.

Agenda: The purpose of this meeting will be to discuss the Cancer Health Disparities Progress Review Group Report.

Place: National Institutes of Health, Building 31, Room 11A03, Bethesda, MD 20892, (Telephone Conference Call.)

Contact Person: Cherie Nichols, Executive Secretary, National Cancer Institute, National Institute of Health, Building 31, Room 11A03, Bethesda, MD 20892, (301) 496–5515.

This notice is being published less than 15 days prior to the meeting due to scheduling conflicts.

Any interested person may file written comments with the committee by forwarding the statement to the Contact person listed on this notice. The statement should include the name, address, telephone number and when applicable, the business or professional affiliation of the interested person.

Information is also available on the Institute's/Center's home page: *deainfo.nci.nih.gov/advisory/joint/htm,* where an agenda and any additional information for the meeting will be posted when available.

(Catalogue of Federal Domestic Assistance Program Nos. 93.392, Cancer Construction; 93.393, Cancer Cause and Prevention Research; 93.394, Cancer Detection and Diagnosis Research; 93.395, Cancer Treatment Research; 93.396, Cancer Biology Research; 93.397, Cancer Centers Support; 93.398, Cancer Research Manpower; 93.399,

FINAL AGENDA

SCIENTIFIC ADVISORY COMMITTEE ON ALTERNATIVE TOXICOLOGICAL METHODS
MARCH 10-11, 2004
HYATT REGENCY HOTEL, BETHESDA, MD

WEDNESDAY, MARCH 10, 2004

8:30 AM	**CALL TO ORDER AND INTRODUCTIONS**	Dr. Jack Dean, Sanofi-Synthelabo, Inc., Chair
8:45 AM	**WELCOME AND REMARKS FROM THE NIEHS/NTP**	Dr. Christopher Portier, NIH/NIEHS
8:55 AM	**WELCOME AND REMARKS FROM THE INTERAGENCY COORDINATING COMMITTEE ON THE VALIDATION OF ALTERNATIVE METHODS (ICCVAM)**	Dr. Leonard Schechtman, NCTR/FDA
9:05 AM	**HOUSEKEEPING**	Dr. Kristina Thayer, NIH/NIEHS
9:10 AM	**UPDATE ON ACTIVITIES OF NTP INTERAGENCY CENTER FOR THE EVALUATION OF ALTERNATIVE TOXICOLOGICAL METHODS (NICEATM) AND THE ICCVAM**	Dr. William Stokes, NIH/NIEHS
10:10 AM	***BREAK***	
10:25 AM	**UPDATE ON ACTIVITIES OF THE EUROPEAN CENTRE FOR THE VALIDATION OF ALTERNATIVE METHODS (ECVAM)**	Dr. Thomas Hartung, ECVAM
10:50 AM	**TOXICOLOGY IN THE 21ST CENTURY: THE ROLE OF THE NATIONAL TOXICOLOGY PROGRAM** • Public Comments • Committee Discussion	Dr. Christopher Portier, NIH/NIEHS
11:50 AM	***LUNCH***	
1:00 PM	**ICCVAM STRATEGIC PLANNING PROCESS** • Public Comments • Committee Discussion	Dr. Leonard Schechtman, NCTR/FDA Dr. Marilyn Wind, CPSC
2:00 PM	**UPDATE ON ANIMAL USE** • USDA: Research Facility Reporting Requirements	Dr. Jodie Kulpa-Eddy, USDA
2:30 PM	**UPDATE ON ICCVAM RECOMMENDED PERFORMANCE STANDARDS FOR IN VITRO DERMAL CORROSIVITY METHODS**	Dr. Amy Rispin, EPA
3:00 PM	***BREAK***	

SCIENTIFIC ADVISORY COMMITTEE ON ALTERNATIVE TOXICOLOGICAL METHODS
MARCH 10-11, 2004
HYATT REGENCY HOTEL, BETHESDA, MD

WEDNESDAY, MARCH 10, 2004

3:15 PM	**VALIDATION OF GENETICALLY MODIFIED MOUSE MODELS** • Public Comments • Committee Discussion	Dr. John Bucher, NIH/NIEHS
4:45 PM	***ADJOURN***	

THURSDAY, MARCH 11, 2004

8:30 AM	**CALL TO ORDER AND INTRODUCTIONS**	Dr. Jack Dean, Sanofi-Synthelabo, Inc., Chair
8:40 AM	**UPDATE OF ILSI/HESI SUBCOMMITTEE'S ACTIVITIES ON IDENTIFICATION OF BIOMARKERS OF TOXICITY**	Dr. Jack Dean, Sanofi-Synthelabo, Inc.
9:10 AM	**ICCVAM NOMINATIONS** • Overview • *In Vitro* Endocrine Disruptor Nominations • *In Vitro* Test Methods to Identify Substances Causing Irreversible Ocular Damage • Other Ocular Toxicity Nominations • Public Comments • Committee Discussion	Dr. William Stokes, NIH/NIEHS
10:10 AM	***BREAK***	
10:30 AM	**UPDATE ON THE IN VITRO VACCINE POTENCY TESTS FOR VETERINARY LEPTOSPIRA VACCINES**	Dr. Jodie Kulpa-Eddy, USDA
11:00 AM	**EVALUATION OF THE UNDER-PREDICTION RATE OF *IN VIVO* DERMAL CORROSIVITY TEST METHODS** • Public Comments • Committee Discussion	Dr. William Stokes, NIH/NIEHS Dr. Joe Haseman, NIH/NIEHS
12:00 PM	**GENERAL SACATM DISCUSSION**	
12:30 PM	***ADJOURN***	

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