Scientific Advisory Committee on Alternative Toxicological Methods

Meeting on December 5, 2002
Crystal Gateway Marriott, Arlington, Virginia

Summary Minutes

The Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) met on December 5, 2002 at the Crystal Gateway Marriott, Arlington, Virginia.

SACATM Members Present

Daniel Acosta, Jr., Ph.D.
Rodger D. Curren, Ph.D.
Jack Dean, Ph.D. (chair)
Nancy Flournoy, Ph.D.
Sidney Green, Jr., Ph.D.
A. Wallace Hayes, Ph.D.
Nancy A. Monterio-Riviere, Ph.D.
Stephen H. Safe, Ph. D.
Carlos Sonnenschein, M.D.
Martin L. Stephens, Ph.D.
Katherine A. Stitizel, D.V.M.
Calvin C. Willhite, Ph.D.

SACATM Members Absent

Alan Goldberg, Ph.D.
Jacqueline H. Smith, Ph.D.
Peter Theran, V.M.D.

ICCVAM Ex Officio Members Present

George Cushmac, Ph.D. (DOT)
Patty Decot (DOD)
Kailash Gupta, Ph.D. (CPSC)
Vera Hudson (NLM)
Joseph Merenda (USDA)
Alan Poland, M.D. (NCI)
Leonard Schechtman, Ph.D. (FDA/NCTR)
Margaret Snyder, Ph.D., (NIH/OD)
William Stokes, D.V.M. (NIH/NIEHS)

NIEHS Staff Present

John Bucher, Ph.D.
Sally Fields
Loretta Frye
Debbie McCarley
Denise Lasko
Christopher Portier, Ph.D.
Mary Wolfe, Ph.D.

Other Federal Agency Staff Present

Richard McFarland Ph.D. (FDA/CBER)

Members of the Public Present

Sara Amundson
Eileen Francis
Thomas Hartung, Ph.D.
Pat Phibbs
Amy Rispin
Troy Seidle
Raymond Tice, Ph.D.

I. Introductions

Dr. Jack Dean of Sanofi-Synthelabo Inc., Chair of SACATM, welcomed everyone to the first meeting of the SACATM. The individuals attending the meeting included SACATM members, the ex officio SACATM members representing the agencies on the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), and NTP/NIEHS program staff. The individuals in the audience then

introduced themselves. Dr. Dean identified the SACATM members who were absent and the ICCVAM agencies not attending.

Dr. Christopher Portier, Director of the Environmental Toxicology Program, National Institute of Environmental Health Sciences (NIEHS), welcomed everyone and on behalf of the Director of NIEHS, Dr. Kenneth Olden, thanked them for attending. Dr. Portier recognized Dr. Thomas Hartung, Head of the European Centre for the Validation of Alternative Methods (ECVAM), and thanked him for participating in the first SACATM meeting. Dr. Portier also thanked the ICCVAM members and the public for attending and invited them to provide comments during the meeting.

Dr. Portier noted the important role of the SACATM in providing advice on the translation of science into policy and regulatory actions. He noted the changing face of toxicology from observational studies and animal models to more complicated paradigms. As these changes occur, they will provide opportunities for new alternative toxicological methods, such as molecular biological techniques, that can reduce, replace, or refine animal use in testing and in regulatory policy. The NIEHS and NTP want to stimulate these activities, while maintaining high quality scientific investigation in order to protect public health.

Dr. Leonard, Schechtman, Chair of ICCVAM, provided brief remarks. He acknowledged the importance of SACATM for providing to ICCVAM advice and direction on its priorities, productivity, resources and efficiency. He gave a brief overview of ICCVAM and noted its role within the Federal government to provide a systematic process for validating methods that reduce, refine, or replace animals in testing and research. This process aids the translation of research and development into tools that regulatory agencies can use to ensure public safety and characterize potential hazards of consumer products or environmental agents. The adoption of new or revised methods is also responsive to animal welfare concerns.

Dr. Mary Wolfe, Executive Secretary, went over housekeeping issues and read the conflict of interest statement to the SACATM.

II. Informational Overviews

A. Overview of NIEHS and NTP

Dr. Christopher Portier, NIEHS, gave an informational overview describing NIEHS and the National Toxicology Program (NTP) and a historical perspective of SACATM and its role as an advisory committee. He briefly outlined the ICCVAM Authorization Act of 2000 (“the Act,” Public Law 106-545) that formally established SACATM and ICCVAM, and outlined the purpose and duties of ICCVAM. He said the importance of the Act is to promote the regulatory acceptance of new or revised scientifically valid toxicological methods that protect human health and animal health while replacing animal tests or refining or reducing the use of animals in testing and to create a formal process for easily incorporating those test methods into the regulatory arena.

He next identified the three specific components of the Act: the ICCVAM, the NTP Interagency Center of the Evaluation of Alternative Toxicological Methods (NICEATM), and the NIEHS Director, and the statutory requirements of each under the law. He then briefly described the organizational relationships of NIEHS, NTP, ICCVAM, NICEATM, and SACATM. Dr. Portier introduced a series of slides indicating where NIEHS and NTP fall within the Federal government.

Dr. Portier described the NIEHS mission, the various programs to carryout that mission, and its organizational structure. He noted that the Environmental Toxicology Program (ETP), which is a part of the NIEHS intramural research program, has responsibility for management of NTP activities. Dr. Portier then delineated some of the NIEHS intramural research activities that relate to alternative toxicological methods, such as computational modeling of biological systems, functional genomics, mechanistic toxicology, mutagenesis, toxicogenomics, and gene-interaction mapping.

He next described the NTP's mission, its organizational structure, and detailed responsibilities of the NTP Program Office. He gave details on the NTP's external advisory groups - the NTP Executive Committee and the NTP Board of Scientific Counselors, and their roles and responsibilities. He also pointed out that SACATM would provide advice to the NTP.

Dr. Portier pointed out that the NTP in concert with the NIEHS' extramural division supports development of alternative toxicological methods and delineated some examples, including *C. elegans* as a developmental neurotoxicology screen, a small business set-aside to develop an *in vitro* renal toxicity screen, the Visible Mouse Project, human biomarker development, and the National Center for Toxicogenomics – an effort to develop microarray technologies for routine screening.

He then reviewed the interactions between NICEATM and ICCVAM, describing the peer review panels and workshops. He noted that ICCVAM and the peer review panels review available published and unpublished data, reports, and all relevant information and make recommendations about the validation status and potential regulatory applicability of test methods. Dr. Portier said the Act requires that the Secretary (or his designee) transmit ICCVAM test recommendations to the appropriate federal agencies. The agencies have 180 days to respond and the responses will be made public.

Dr. Portier went over the structure of SACATM and detailed its charge. He pointed out that ICCVAM members are non-voting *ex officio* members of SACATM. Dr. Portier briefly outlined some of the issues that might come before SACATM, such as the development of new assays and guidelines for their use, recommendations made by ICCVAM based on the validation of those assays, the identification and evaluation of priorities and directions for ICCVAM and NICEATM, and the procedures and methods used by NICEATM, ICCVAM, NTP and NIEHS in managing validation-related activities. He pointed out that SACATM would also receive public input and provide advice on the importance of that input relative to the ICCVAM processes.

Finally, Dr. Portier identified NTP Program Office staff and recognized the efforts of Dr. William Stokes, Director, NICEATM, and his staff.

1. Discussion

The SACATM asked for copies of Dr. Portier's slides and he replied that they would be sent. Dr. Acosta asked whether Congress appropriated funds for ICCVAM-NICEATM activities. Dr. Portier replied no. Dr. Stephens asked if SACATM's advice could also be directed at the NIEHS research and development activities described by Dr. Portier. Dr. Portier said NIEHS and NTP would be glad to give formal presentations about their activities and in addition, pointed out that other agencies – FDA, Environmental Protection Agency (EPA), Department of Defense, Department of Energy, and National Cancer Institute - also have similar types of activities and suggested that they might also give formal presentations to the SACATM. The SACATM asked that the agencies represented on ICCVAM provide information at a future meeting about their agencies' activities on alternative toxicological methods, including both extramural and intramural activities when appropriate. Dr. Schechtman said the National Center for Toxicological Research (NCTR) of the FDA would be willing to participate.

B. Overview of NICEATM and ICCVAM

Dr. William Stokes, NIEHS, provided an overview of NICEATM and ICCVAM, detailed the background and history of each group, and presented examples of recent ICCVAM test method evaluation activities. Dr. Stokes noted that NIEHS initially established ICCVAM as an ad hoc interagency committee in 1994 in response to specific mandates in the NIH Revitalization Act that required NIEHS to develop criteria for the validation and regulatory acceptance of alternative toxicological testing methods, and to develop a process to achieve the regulatory acceptance of scientifically valid methods ICCVAM became a standing committee in 1997 and a permanent interagency committee under NICEATM with passage of the ICCVAM Authorization Act of 2000 (Public Law 106-545) The ICCVAM/NICEATM web site http://iccvam.niehs.nih.gov has all historical documents related to ICCVAM. The 15 member agencies of ICCVAM include Consumer Products Safety Commission, Department of Agriculture, Department of the Interior, Department of Transportation, EPA, Occupational Safety and Health Administration, Agency for Toxic Substances and Disease Registry, DOD, DOE, NIOSH, FDA, NCI, National Library of Medicine, National Institutes of Health, and NIEHS. Dr. Stokes identified the specific purposes and duties of ICCVAM as mandated in the Act.

Dr. Stokes next described NICEATM and its responsibilities and duties. The NICEATM administers and provides committee management for the ICCVAM; assures ICCVAM compliance with applicable provisions of the Act; provides operational and scientific support for the ICCVAM, its Working Groups, and scientific panels; organizes and convenes workshops, expert panels and peer review panels on behalf of and in collaboration with ICCVAM; promotes communication with stakeholders; and facilitates development of partnerships with stakeholders and test method developers. He then

defined the goals of NICEATM and ICCVAM which are to promote the scientific validation and regulatory acceptance of new alternative test methods that are more predictive of human health and ecological effects than currently used methods and to refine, reduce, and replace animal use where scientifically feasible.

Dr. Stokes briefly outlined the steps in the test method evaluation process and the responsibilities of NICEATM, ICCVAM, the interagency working groups, and the scientific peer review panels. He noted that once an expert panel review is completed, the ICCVAM working group reviews the panel's report and drafts test method recommendations. The ICCVAM then reviews these recommendations, makes appropriate changes, and transmits this information to the appropriate federal agencies through the Secretary. The agencies make decisions about the acceptability of a test method according to their own mandates and submit their responses to ICCVAM. According to the Act, these agency responses will be made public.

Dr. Stokes pointed out that NICEATM also convenes workshops and expert panel meetings for test methods that have not undergone complete validation. The objectives of these meetings may include the identification of research and/or model development efforts needed to improve a test method, identification of test methods that should undergo further development or validation, and identification of additional studies necessary to complete validation of a test method

Dr. Stokes summarized the test methods evaluated to date by NICEATM and ICCVAM.

- Three methods have undergone formal independent scientific peer review. ICCVAM recommendations on these methods have been forwarded to the agencies and the test methods have been or are in the process of being adopted by national and/or international regulatory authorities. They include:
  - Local Lymph Node Assay for assessing allergic contact dermatitis (dermal hypersensitivity) which provides for reduction and refinement of animal use and eliminates potential unrelieved pain and distress that was previously associated with the older assay for which it can be substituted. The LLNA has now been adopted by U.S. agencies and an OECD international test guideline has also been adopted. An international training workshop on implementation of the LLNA was held in 2001 to facilitate use of the test method.
  - Corrositex®, an *in vitro* test method for assessing dermal corrosivity potential of chemicals and products. This test method has been accepted by DOT for transportation hazard assessments and by other agencies as a screening assay for use in a tiered testing strategy for dermal irritation/corrosivity assessments.
  - The revised Up-and-Down Procedure for acute oral systemic toxicity, which significantly reduces the number of animals needed for this product safety testing requirement. The UDP has now been adopted and recommended by EPA as the preferred test method for acute toxicity determinations and has been adopted by OECD as an official test guideline for international use. An international training implementation workshop for both *in vitro* and *in vivo* methods for acute oral systemic toxicity was held in February 2002.

- Three other methods for assessing dermal corrosivity, which have been evaluated and recommended, used an expedited review process because the methods had undergone independent validation studies by ECVAM. These include:
  - Epiderm, Episkin, and a rat skin transcutaneous electrical resistance method
- An international workshop on *in vitro* methods for assessing acute systemic toxicity was held in October 2000. Attendees at the workshop developed recommendations on research, development, and validation activities for screening methods, toxicokinetic methods, target organ toxicity methods, and chemicals for use in validation of these methods.
- Two expert panel meetings were conducted to assess the validation status of available test methods for which there is existing, but incomplete validation:
  - Frog Embryo Teratogenesis Assay (FETAX) is a proposed developmental screening assay for toxicity for which recommendations were provided for further standardization necessary to improve its reproducibility.
  - Four types of *in vitro* endocrine disruptor assays proposed for using in the EPA endocrine disruptor tier 1 screening battery have been assessed. NICEATM prepared comprehensive background review documents on these methods to facilitate evaluation of their validation status. This included data from over 4000 tests conducted using at least 137 different protocols to evaluate over a 1000 different chemicals. Dr. Stokes said Dr. George Daston would provide a full description of the expert panel review later in the meeting. [Due to unfavorable weather conditions, Dr. Daston was unable to attend the meeting and give a presentation on the peer review meeting for these assays].

Dr. Stokes noted other related activities of ICCVAM and NICEATM, including co-organizing and participating in the OECD International Conference on Validation and Regulatory Acceptance of New and Updated Methods in Hazard Assessment, co-organizing and participation in the First International Symposium on Regulatory Testing and Animal Welfare in 2001, and significant contributions to the OECD guidance document – “Endpoints for Experimental Animals used in Safety Evaluations,” published in 2000.

Dr. Stokes pointed out that ICCVAM addresses issues in addition to test method evaluations. Some of the current items under discussion include identifying minimum performance standards for test methods that ICCVAM reviews, preparing an OECD guidance document for applying GLPs to *in vitro* toxicity studies, and undertaking a retrospective review of *in vivo* dermal irritation and corrosivity data to estimate the false negative rate of the currently accepted methods.

Dr. Stokes recognized the contributions of the other two NICEATM staff, Ms. Debbie McCarley and Loretta Frye, the staff contributions from the participating ICCVAM agencies, and the ILS staff on the NICEATM support contract. He acknowledged their important role in making NICEATM and ICCVAM activities successful.

III. ICCVAM Validation and Acceptance Criteria

Dr. Stokes next discussed the criteria for validation and acceptance of toxicological test methods developed by the ad hoc ICCVAM and interested stakeholders. These criteria are described in the 1997 report, "Validation and Regulatory Acceptance of Toxicological Test Methods: A Report of the ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods." Using a series of slides he outlined the criteria that need to be addressed for proposed new methods and the criteria for test method acceptance. He stated that scientific validation is a process that determines the usefulness and limitations of a test method for a specific purpose. He emphasized that validation studies do not always demonstrate that a test method is valid for a proposed use, in that it may not have adequate accuracy or reproducibility. He pointed out that in order for the test method to be used, the test method must have undergone adequate validation studies, be determined to be scientifically valid for its proposed use by an independent scientific peer panel, be recommended by ICCVAM for specific regulatory purposes, and be accepted by the appropriate regulatory authorities. The overarching requirement for regulatory acceptance is a determination that the proposed use of data from the new method will provide for a comparable or better level of protection of human health or the environment than the current method or approach. He concluded by mentioning the availability of guidance prepared by ICCVAM for test developers considering submissions to ICCVAM. This document, "Evaluation of the Validation Status of Toxicological Methods: General Guidelines for Submissions to ICCVAM," was developed to facilitate the efficient and effective review of test methods and is available on the ICCVAM/NICEATM website.

1. Public Comment

Sara Amundson from the Doris Day League said her organization was instrumental in passage of the ICCVAM Authorization Act of 2000. She said a letter was sent to the NIEHS, which was distributed to the SACATM, raising concerns about the development of SACATM and the charter. The charter identifies three areas on which the SACATM will provide advice to NICEATM. In the first area that deals with priorities and opportunities for alternative test methods, Ms. Amundson noted that the replacement of methods is omitted and asked that the charter be amended to reflect NIEHS' commitment to all three Rs – replacement, refinement, and reduction of test methods that use animals. She pointed out that the Authorization Act requires uniform criteria be used in assessing all new, revised, or alternative test methods and that any proposed method should be scientifically validated before an agency incorporates it into its testing paradigm. Ms. Amundson expressed concern about funding for ICCVAM activities in her remarks and asked the SACATM to consider this issue.

[In her presentation, Ms. Amundson noted that the copy of the charter provided as a meeting handout was incomplete. Following lunch, complete copies of the SACATM charter were made available to the SACATM, ICCVAM and observers.]

Troy Seidle from the People for the Ethical Treatment of Animals (PETA) addressed the SACATM and provided a hardcopy PowerPoint presentation as a handout. He said from 1997-2001Europe has taken the lead in non-animal test method development, validation and regulatory acceptance. He felt that the U.S. Federal agencies do not have a cohesive, organized, and coordinated strategy to develop and validate endpoint-specific replacement methods and PETA believes NICEATM should serve as the coordinator for this activity. He supported the concerns raised by Ms. Amundson regarding funding for NICEATM-ICCVAM activities and asked SACATM to address this issue. He said PETA is not pleased with the U.S. decision to only accept the three corrosivity tests as methods to screen for positive results noting that ECVAM and the European Union accepted the methods as standalone replacements. He also mentioned that a recommendation from the OECD Stockholm meeting in February was for a workshop on the collection of human data. It is envisioned that this database could be used for validation purposes. Mr. Seidle said PETA requested a review of this matter in a letter to the NIEHS Director and he asked if the status of that review could be discussed at this meeting.

2. Discussion

A SACATM member asked for additional details about the proposed workshop on human data. Mr. Seidle said his comments were based on participating in a non-governmental organization (NGO) teleconference briefing prior to the joint OECD meeting in Stockholm.

Several members asked about federal funding for alternative methods. In reply, Dr. Portier explained the budget request process at the NIEHS and added that across the realm of new technologies, such as molecular techniques, the NIEHS has requested additional funding. He added that budgetary issues are not the purview of SACATM; however, he welcomed getting the committee's input about the science being supported and its future directions. The Chair felt an important role for SACATM would be to examine the current efforts focused on development and validation of alternative methods with consideration given to the total interagency effort and to help set priorities for future activities.

Dr. Green complimented Dr. Stokes on the ICCVAM-NICEATM web site and its usability. He asked if a replacement method were accepted by an agency would the agency still accept data using the old method. Dr. Stokes deferred to the ICCVAM regulatory agencies to answer this question. Dr. Schechtman replied that there are a number of factors associated with the acceptance and implementation processes for a method that are considered, such as its scientific validity, technical feasibility, and applicability for use. If an agency accepted the alternative method, there could be a phase-in period for it, a period of overlapping activity in which data from both the old and the new methods are received, followed by a phase-out period for submission of data using the old test method. In reply to a question, Dr. Schechtman said the incentives for an industry to adopt the alternative method might include improved hazard assessment, animal welfare, increased efficiency of the replacement method, and cost-savings. Dr. Stokes pointed out that the Animal Welfare Act (AWA) and its

regulations require consideration of alternatives and the Institutional Animal Care and Use Committees reviewing proposals using AWA-covered species would require justification for not using an available alternative method. The Public Health Service Policy on the Humane Care and Use of Laboratory Animals similarly requires consideration of alternatives prior to the use of laboratory animals for research, testing, or educational purposes. Dr. Stokes said future test method evaluations would include developing minimum performance standards so that other companies that might want to develop a similar test would know what performance would need to be achieved in or order to be considered acceptable.

IV. Current Scientific Directions of the European Centre for the Validation of Alternative Methods (ECVAM)

Dr. Thomas Hartung, the Unit Head, for ECVAM, provided a presentation about ECVAM, its roles and responsibilities, and the potential for collaboration with ICCVAM. He noted that ECVAM is involved in both the development and validation of replacement methods. Dr. Hartung described where ECVAM falls within the Joint Research Commission (JRC) and the European Union. He described the research interests of ECVAM, the roles of the ECVAM Task Leaders, and the ECVAM Scientific Advisory Committee (ESAC).

Dr. Hartung described ECVAM's research and study efforts in neurotoxicology, reproductive toxicology, stem cell biology, cancer, chronic toxicity and metals. He described the role of task forces and workshops to the effectiveness of ECVAM and identified future workshops on embryotoxicity and the validation of QSAR. ECVAM is developing a database of 150 scientifically evaluated methods that will include information such as standard operating procedures. He noted the importance of good laboratory practices and supports development of guidance documents on the application of GLPs to *in vitro* testing methods.

Dr. Hartung described some new duties and goals of ECVAM, such as assembling more task forces and holding more workshops, strengthening the role of task leaders, and making the ESAC more independent. He also identified some issues being addressed by ECVAM such as international harmonization of testing methodologies, validation of testing strategies, availability of poor *in vivo* data, and time pressures to meet the requirements for alternatives in the areas of testing chemicals and cosmetics. Dr. Hartung emphasized his desire to continue international harmonization through OECD and maintain strong ties with ICCVAM and NICEATM. He closed by introducing the new ECVAM web site http://ecvam-sis.jrc.it.

1. Discussion

Dr. Willhite asked how SACATM might work through Drs. Stokes and Schechtman to contribute to the proposed collaborations. Dr. Hartung said he envisions the advisory committees having regular observers attend each other's meetings. He also sees

ICCVAM and NICEATM working with ECVAM, collaborating on validation projects, exchanging information, and holding joint workshops. He referred to the recently published articles by Drs. Stokes and Schechtman that outline current and potential collaborations, and said he agrees with their suggestions. Several members praised Dr. Hartung, Dr. Schechtman, and Dr. Stokes for moving forward with improved communication and cooperation between their groups. Dr. Stitzel had a question about building an international pharmaceutical regulation organization. Dr. Hartung replied that he hoped to establish close collaboration with the pharmaceutical companies.

V. ICCVAM and ECVAM – Interactions and Collaborations

Dr. Schechtman, FDA/NCTR, said ICCVAM's vision is to work synergistically with ECVAM to evaluate the scientific validity of new and alternative methods that will address the 3Rs (replacement, refinement and reduction of animal use in testing) and to promote international harmonization and adoption of ECVAM-ICCVAM-recommended methods. He described some of the past interactions and collaborations. He broadly defined opportunities for exploiting the two groups' common missions, goals, duties, and visions. He mentioned possible areas of collaboration including methods development and validation efforts and joint ECVAM-ICCVAM workshops, seminars, and study sections. Strategies aimed at carrying out these alliances might include partnering with ECVAM to harmonize validation evaluation processes, working to reduce redundancy, sharing expertise, defining a streamlined evaluation process for expedited review of methods previously reviewed by ECVAM, promoting reciprocal participation in ECVAM- and ICCVAM-sponsored events, working to standardize methods and processes that both ECVAM and ICCVAM employ, maintaining open and continual dialogue between the two groups, and leveraging resources between ICCVAM and ECVAM toward co-sponsorship of workshops, validation efforts, and research and development efforts of mutual interest.

Dr. Schechtman identified some similarities and differences between ESAC – the ECVAM Scientific Advisory Committee and SACATM and he supported reciprocal exchange of *ex officio* liaisons between the two committees. He said ICCVAM and ECVAM have already initiated some collaborations through 1) ESAC making formal statements on ICCVAM validated methods such as the Local Lymph Node Assay and Corrositex, 2) ICCVAM putting an expedited review process in place to evaluate ECVAM-recommended test methods such as the *in vitro* methods for assessing dermal corrosivity of chemicals, and 3) ICCVAM-ECVAM reciprocal participation in events sponsored by the other respective group. In closing, Dr. Schechtman noted the importance of the current and continued interactions between ECVAM and ICCVAM.

1. Public Comment

Ms. Amundson, Doris Day Animal League, brought up instances where scientific conclusions by ECVAM do not mirror decisions by ICCVAM and mentioned skin corrosivity as an example. Dr. Hartung responded that it would be important to clarify

such issues and if possible work toward a compromise. Dr. Schechtman added that hopefully by working together early in the process, sharing data, and jointly establishing the acceptance criteria for a method, the chance for divergent outcomes could diminish. Dr. Stokes concurred reinforcing the need to share the data used to make decisions on acceptance criteria, and to make this process open and transparent so that everyone has access to the data and can understand the basis for such decisions.

Mr. Seidle from PETA added that the collaborations between ECVAM and ICCVAM should not be limited to validation studies, but also extend to research and development of methods. He also supported formalization of the coordination of research between ECVAM and NICEATM and other U.S. and international agencies.

2. Discussion

Dr. Hartung suggested that an initial ICCVAM-ECVAM collaboration might be a workshop to fine tune differences in the validation procedures and the development of independent statements by the two groups.

A question was raised whether SACATM has a role in methods development as well as validation. Dr. Schechtman replied that the SACATM does play a role in advising on methods development. A subsequent question was asked about resources available across the agencies and the SACATM's access to that information for use in helping set priorities. Dr. Dean remarked that the agencies would be invited to give presentations on their activities to SACATM. Dr. Stephens and other members endorsed collaborations between ICCVAM and ECVAM and he supported receiving more information about the budget.

Dr. Hartung was asked to clarify the role of ESAC and some of the differences between SACATM and ESAC. He said ESAC's role is to bring representatives from the 15 European Union (EU) member states together to harmonize the efforts being undertaken by ECVAM. One specific difference between ESAC and SACATM is that ESAC makes formal recommendations on the scientific validity and acceptability of test methods to ECVAM and in this way promotes the acceptance of validated methods in the EU. Dr. Hartung estimated that approximately $60-$70 million was spent on the development of alternatives between 1998 and 2002 by the Directorate General Research within the EU. He added that ECVAM's budget for the same four-year period was about $36 million.

In response to a question from Dr. Willhite, Portier clarified that SACATM would be asked to comment on the priorites for which methods to move through the validation process based upon the methods before NICEATM and the available resources. The SACATM might also be asked to comment on the validation process. Dr. Stokes said the independent expert panels convened by NICEATM assess the validation status of a method, and added that a SACATM member might be invited to participate on a panel for an area of relevant scientific expertise.

Dr. Stitzel expressed concern that the [ECVAM] validation management committee has too much authority in deciding how a method's validation is conducted. She hoped that the initiation of stronger ICCVAM-ECVAM collaborations would result in greater public input on this process including comments by agencies that would ultimately determine the method's acceptability. Dr. Schechtman replied that such issues are already being addressed and acknowledged the importance of making the validation process more transparent. Dr. Curren stressed that the validation process should be based on the scientific validity of the method. He added that for setting up the validation process for a replacement method, it would be helpful if the agencies would provide information about how the standard method performs as it is currently being used in the regulatory process.

VI. ICCVAM Test Method Submission, Nomination, and Prioritization Process

Dr. Stokes, NIEHS, gave a presentation on the process for submission of test methods for consideration by the ICCVAM and a proposed process for nomination and prioritization of submissions. He noted that the ICCVAM Authorization Act of 2000 specifies that a test method submission must identify the specific regulatory mandate that the method addresses and provide evidence of its scientific validity. ICCVAM has developed guidelines for test method submissions that outline the basic elements required in a submission. Criteria involved include assessment of reliability, repeatability, reproducibility, accuracy, adherence to GLPs, and animal welfare considerations. He pointed out that the guidelines are available in both printed text and on the ICCVAM/NICEATM web site.

Dr. Stokes stated that there are currently no test method submissions in the review process. Some test methods that may be submitted in 2003 include estrogen receptor and androgen receptor binding and transcriptional assays proposed as mechanistic assays for tier 1 endocrine disruptor screening battery, and Epiocular™ and human corneal epithelial model – both *in vitro* methods proposed for assessing ocular irritation potential of surfactants and surfactant containing materials. He talked about other test methods that might be considered as nominations, but would not be considered full submissions because all of the information required by the guidelines is incomplete. In such cases, significant resources might be required to organize and prepare the documentation necessary for an evaluation of the validation status of the test method.

Dr. Stokes next outlined the proposed criteria for prioritizing test method nominations and submissions and described the proposed step-wise process for ICCVAM test method submission, nomination, and prioritization. He pointed out that ICCVAM would recommend a draft priority for evaluation, conduct of a validation study, or other relevant activity. The SACATM would receive this information along with any public comments and make a recommendation on the priority. ICCVAM would consider these comments and then finalize their priority. The NICEATM would prepare an estimate of the resources necessary for the recommended activity, such as a validation study. The

Director of the Environmental Toxicology Program would make a decision on resource requests. Dr. Stokes closed by inviting comment from the SACATM on its proposed role of reviewing and commenting on test method submissions and nominations in order to assist the program in setting priorities.

1. Public Comments

Troy Seidle from PETA asked for clarification about the process and whether nominated methods would have to wait for a scheduled SACATM meeting or would there be a Federal Register notice and opportunity for public comment. He also asked if NICEATM would solicit nominations of test methods from the public in addition to Federal agencies.

2. Discussion

Dr. Portier clarified that the resource allocations would not necessarily come just from NIEHS, but could be shared with other agencies interested in a specific test method through interagency agreements. Dr. Hayes raised concern about the paucity of test method submissions to the NICEATM and asked if ICCVAM agencies might stimulate this process by identifying areas where alternatives are needed and then publicizing that information.

In response to questions, Dr. Stokes answered that the proposal is that SACATM would be asked to comment on nominations and submissions at their meetings, and information about the test method nominations would be published in the Federal Register at the time the SACATM meeting is announced. He added that the nomination process would be open and NICEATM would accept test method nominations from anyone. Dr. Portier added that if this process were implemented, NICEATM would routinely solicit nominations through the Federal Register in addition to seeking input from Federal agencies. He added that SACATM would review all nominations with meetings being held 1-2 times annually.

Dr. Stitzel asked whether SACATM would be asked for input on setting priorities for more than test methods, e.g., provide comment on areas where additional research is needed or possibly a workshop. She also asked whether there would be a public comment period on a test method. In reply, Dr. Portier said there would be opportunity for public comment and added that NIEHS and NTP would take SACATM's recommendations for R&D seriously in examining their research activities.

Dr. Safe suggested some additional considerations to add to the list for evaluating submissions or nominations. These include giving special consideration to nominations linked to current activities of ICCVAM agencies, OECD, or similar bodies that would facilitate leveraging of resources and to those test methods with immediate utility for ongoing testing activities. He asked how a test method using animals would be addressed and evaluated. Dr. Stokes said for such a method, NICEATM would evaluate whether it is more predictive of the adverse health effect than the current method. In response to a question from Dr. Green, Dr. Stokes said the NICEATM began accepting test method submissions in 1998 and to date there have been no

competing submissions that required prioritization for resources. Dr. Green commented that in setting priorities, the public health significance of a test method should be given careful consideration. Dr. Stokes agreed that the potential to benefit or otherwise improve public health should definitely influence a test method's priority

Dr. Dean asked SACATM for its comment on the following question – How might SACATM effectively and efficiently be included in the process of reviewing test material submissions and aid NICEATM in setting priorities for limited resources?

Dr. Stitzel supported the proposed process, but also endorsed SACATM looking more broadly at helping set priorities for other ICCVAM activities. Dr. Flournoy suggested a pre-proposal submission step be added where comments about the design and analysis being proposed for the test method evaluation could be provided to the developers early in the process to ensure that the proposed process and analysis are scientifically sound and innovative. Dr. Stokes agreed with this, and noted that ICCVAM has interacted with test method sponsors in the past during the design phases and encourages these interactions. Dr. Willhite pointed out that refinement of methods should be encouraged also, if changes can be made to reduce animal use and the method remain scientifically sound. Drs. Curren and Stitzel supported Dr. Flournoy's suggestion of looking at the statistical methodology being used in the evaluation, and Dr. Stitzel suggested the possibility of a test methods workshop to address Dr. Willhite's suggestions`.

Dr. Acosta stressed the idea of gaining a better understanding of available resources so the SACATM could better advise on plans for symposia and workshops. Dr. Dean suggested that ICCVAM might catalyze industry groups to get involved in supporting the validation of test methods in particular areas. Dr. Stephens supported this concept and suggested that the effort toward test method development and validation should be more proactive. Dr. Hayes suggested that ICCVAM and ECVAM might collaborate to identify priorities areas and then seek tests that fit the categories. Dr. Stitzel felt that instead of focusing on specific tests, she suggested that ICCVAM and ECVAM might collaborate on identifying and addressing basic research questions that need to be addressed about the use and application of data from *in vitro systems* for risk assessment.

Dr. Portier noted that changes are needed in the proposed process and offered a summary of the committee's comments.

- Broadly seek nominations from outside entities, including other Federal agencies
- Don't set priorities strictly based upon individual test methods, but look more broadly at the scientific questions that need to be addressed relative to use of data in risk assessment.
  - Workshop can aid in addressing what issues are important
- Consider test methods that address animal welfare issues, not just those applicable to regulatory issues.
- Prioritization of test method submissions should be linked to both the development and analysis of the methods.

- Test method nominations and submissions should be brought to SACATM expeditiously to keep the process moving.

VII. *In Vitro* Acute Toxicity Testing Methods

A. International Workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity: Tasks and Recommendations

Dr. Stitzel, SACATM member, presented an overview about this meeting sponsored by ICCVAM and EPA in October 17-20, 2000. The impetus for the workshop was two-fold: 1) a series of recently published studies suggested that *in vitro* methods could predict acute toxicity with a fair degree of accuracy when compared with human data and 2) a series of papers from Germany proposed that *in vitro* methods should be used to set starting doses for *in vivo* acute toxicity studies.

Dr. Stitzel outlined the workshop objectives 1) to review the status of *in vitro* methods for screening, toxicokinetic parameters, and organ specific toxicity; 2) to identify methods ready for prevalidation or validation; 3) to recommend validation study designs; 4) to identify reference chemicals for the validation studies; and 5) to identify priority areas for research. The workshop included plenary presentations, four breakout groups, opportunities for public comment, and a final report from each breakout group. She said the short-term goal was to develop a way to estimate rodent LD50s using *in vitro* data, initially by using *in vitro* data to reduce animal numbers and eventually to replace animals. The long-term goal was to be able to predict human toxicity using *in vitro* acute toxicity testing.

In a series of slides, Dr. Stitzel briefly discussed the four breakout groups, their members and recommendations.

- Group 1 - In Vitro Screening Methods: they addressed the use of *in vitro* screening methods to estimate *in vivo* toxicity. They proposed a strategy that included *in vitro* tests employing human cells and the integration of these data with information based on physical/chemical parameters to estimate starting doses for *in vivo* studies. They recommended a prevalidation study to evaluate various cell types, exposure periods and endpoints as predictors of acute toxicity. Long-term they recommended development and validation studies of human *in vitro* methods for predicting human acute toxicity integrating the approaches suggested by Groups 2 and 3.
- Group 2 – Toxicokinetic Determinations: this group discussed the role of *in vitro* methods for estimating toxicokinetic parameters needed to assess acute *in vivo* toxicity. They developed a chemical triage strategy and recommended research and validation efforts for tools to estimate metabolism and clearance.
- Group 3 – Specific Organ Toxicity and Metabolism: they examined *in vitro* methods for assessing target organ toxicity and mechanisms. They recommended a 5-step screening process – 1) physical/chemical characterization and biokinetic modeling, 2) basal cytotoxicity, 3) metabolism-mediated toxicity, 4) energy metabolism and 5) epithelial barrier function tests.

- Group 4 – Chemical Data Sets and Validation of *In Vitro* Toxicity Tests: they addressed chemical data sets required for validation of acute *in vitro* toxicity tests. Their recommendations were 2-fold 1) develop a rodent toxicity database that would have a primary set of reference chemicals from which subsets could be used for validation studies of test methods or prediction models and 2) develop a human database for use in comparing data from *in vitro* studies.

B. ICCVAM Evaluation of *In Vitro* Methods for Assessing Acute Systemic Toxicity

Dr. Stokes, NIEHS, provided a description of the initiatives that have been undertaken by ICCVAM and NICEATM to implement some of the recommendations of the workshop. These activities include:

- publishing the workshop report,
- preparing ICCVAM recommendations on the development and use of in vitro methods for assessing acute systemic toxicity ,
- preparing a guidance document on how to use in vitro methods to estimate starting doses for in vivo acute systemic toxicity studies,
- holding a training and implementation workshop for the *in vitro* cytotoxicity methods, and
- initiating a NICEATM-ECVAM validation study on the highest priority methods recommended by the workshop experts.

Dr. Stokes said 110 participants from 9 countries attended the workshop on October 17-20, 2000 in Arlington, Virginia. The workshop report is posted on the ICCVAM/NICEATM web site (http://iccvam.niehs.nih.gov). He presented a diagram of the short-term and long-term strategies developed at the workshop. Following the workshop, ICCVAM reviewed and endorsed the workshop report and developed ICCVAM recommendations that included the following:

Current uses of acute toxicity tests

- "Cytotoxicity assays can be useful tools in setting starting doses for *in vivo* assessment of acute oral toxicity"
- "Using *in vitro* approaches could reduce animal use for acute toxicity determinations"

Research, development and validation of these tests

- "ICCVAM concurs with the workshop recommendation that near-term validation studies should focus on two standard cytotoxicity assays: one using a human cell system and one using a rodent cell system."
- "Longer-term activities should be directed at improving *in vitro* systems that provide information on biokinetics, metabolism, and organ-specific toxicity. These additional tests will be necessary to facilitate reasonably accurate predictions of LD50s, signs and symptoms associated with toxicity, and pathophysiological effects."

Dr. Stokes described the "Guidance Document: Using *In Vitro* Data to Estimate *In Vivo* Starting Doses for Acute Toxicity" prepared by NICEATM in conjunction with three of the invited workshop experts, Drs. Rodger Curren, Manfried Liebsch, and Julia Fentem. The document provides standardized protocols for two cytotoxicity methods - one using a rodent cell line of 3T3 murine fibroblasts and the other using normal human keratinocytes, and algorithms for converting *in vitro* data into an estimated starting dose based on a prediction model developed by ZEBET. It is estimated that using *in vitro* data to estimate starting doses can further reduce the number of animals for an individual acute toxicity study by 30-40%, and for highly toxic chemicals will reduce the number of animals that die or require euthanasia during the study.

He then discussed the workshop, Putting Oral Toxicity Testing Guidelines into Practice: a Training Workshop, held February 19-21, 2002 at the NIH and organized by the ILSI Risk Sciences Institute, ICCVAM, NICEATM and EPA. The workshop was well attended with 122 participants from 11 countries. The program included plenary talks and breakout sessions on the Up-and-Down procedure, the acute toxic class method, the fixed dose procedure, *in vitro* methods, and humane endpoints.

Finally, Dr. Stokes briefly described the joint NICEATM-ECVAM validation study to evaluate two *in vitro* basal cytotoxicity methods, which was identified as the highest priority near-term activity by ICCVAM and the expert scientists at the 2000 workshop. The study is being supported by NIEHS, EPA, and ECVAM. The study's objectives are 1) to standardize and assess the usefulness of two *in vitro* basal cytotoxicity methods for estimating rodent oral LD50 values for each of the United Nation's five globally harmonized classification scheme (GHS) hazard categories and identifying those that will not require a hazard classification, and for estimating human LD50s; 2) to determine to what degree dose selection based on these *in vitro* data can reduce animal use and/or animal mortality; and 3) to generate a database that can be used to support the development and validation of the additional *in vitro* methods that will be needed to increase the accuracy of *in vitro* predictions of acute toxicity. Dr. Stokes identified the groups involved with this project, which include a NICEATM-ECVAM study management team, an NTP Project Design and Evaluation Team, multiple advisory groups, two U.S. laboratories, and a EU laboratory. He discussed the design of the validation study that includes three study phases. The first two phases will focus on further standardization of the protocols in order to minimize variation and maximize intra- and inter-laboratory reproducibility. Dr. Stokes invited the SACATM to provide comments on the acute toxicity workshop's recommendations and the ICCVAM-NICEATM activities undertaken in response to those recommendations.

1. Public Comments

Troy Seidle from PETA stressed the need for research into the translation of metabolism assays into tests useful for regulatory purposes. He supported a joint US-European venture.

2. Discussion

Dr. Acosta asked whether any of the 72 chemicals selected for testing have to be metabolized to show toxicity noting that a similar study conducted by FRAME in the 1970s had found that metabolism was required of a number of the ones tested before toxicity could be shown. In response, Dr. Stokes said some of the chemicals require metabolism, but was unsure how many. It was noted that the FRAME laboratory is part of the NICEATM-ECVAM validation study.

Dr. Curren said his laboratory is participating in the validation study. Dr. Wolfe clarified that they are aware of this involvement and a special waiver was requested for Dr. Curren's participation on the SACATM. When the validation study data are presented to SACATM, Dr. Curren will not serve as a primary reviewer and will recuse himself from participating in any decisions by SACATM regarding this issue.

Dr. Hayes commended Drs. Stitzel and Stokes on their presentations. He wondered why this study was not included in the list of current activities and Dr. Stokes replied that he only included items for 2003. Dr. Hayes asked if EPA had any feedback about acute toxicity test results from any of the high production volume chemicals noting that this information would be useful to the current validation study. He thought consideration should be given to the recommendations from Groups 2,3, and 4 of the *in vitro* workshop and supported compiling the reference chemical database and having a repository of chemicals for use in validation studies. Dr. Stokes acknowledged Dr. Hayes' comment about listing the validation study among ICCVAM's activities and added that the validation study is being undertaken because it was thought that validating these assays could have some near-term effect on reducing animal use for the High Production Volume Program. Dr. Stokes said Group 4's recommendation was considered in establishing the set of 72 chemicals being used in the current validation study; they underwent thorough review and selection.

Dr. Tice, ILS, explained the process for compiling the chemical list and noted that the selection of chemicals was based on work comparing LD50 and *in vitro* toxicity by the German group. Additional chemicals were added to address metabolism issues. He said they tried to identify the best studies for determining the LD50 *in vivo* and considered mechanism of action when that information was published. Each chemical essentially has its own dossier. Dr. Willhite noted that variability among the data could be due to assay differences among laboratories and Dr. Tice replied that they considered vehicle, assay conditions, and species and strain in examining the studies. Dr. Tice said that the protocol for the validation study does not include the use of supplemental metabolic activation for the *in vitro* studies. Dr. Portier said that the NTP included Dr. Joe Haseman as part of this project to ensure that the statistical analyses were in place prior to initiating it.

Dr. Stitzel supported the comments about needing a reference chemical database for the various endpoints and suggested that the agencies could facilitate this effort by helping identify the classes of chemicals to include. She further suggested that a workshop with the pharmaceutical companies to share information about metabolism

assays would be instructive. Dr. Dean thought participation by those companies would be feasible.

Dr. Flournoy asked where the guidance document stands in terms of statistics and Dr. Stokes said the guidance document simply covers two currently available *in vitro* methods that are considered fairly standardized and reproducible. He said the validation study would examine the current algorithm for converting *in vitro* data into estimates of the *in vivo* LD50and its usefulness.

Dr. Stokes then briefly outlined the rationale for the current validation study. Basically it is being undertaken to verify the reported relationship between *in vitro* basal cytotoxicity and *in vivo* LD50. The study design involves the standardization and use of 2 *in vitro* basal cytotoxicity tests in 3 laboratories to evaluate a standard set of reference chemicals. The results will provide baseline values that can serve as the basis for identifying and evaluating the other types of additional *in vitro* tests that will be necessary to accurately predict LD50s. It is anticipated that this will require test methods to assess the extent that metabolic activation or inactivation will occur, whether certain CNS, cardiac or other receptors are affected, whether there is specific organ toxicity induced, and whether there is selective passage across critical membranes such as the blood-brain barrier. Dr. Acosta made a number of comments: change the title to include LD50, appropriately reference the experts who developed the Neutral Red Test, and reexamine the literature on target organ toxicology. He was concerned about the resources for this project and felt that a project should be carefully evaluated in terms of the scientific question(s) being addressed and its impact on the Federal agencies before resources are allocated.

Dr. Safe questioned the value of doing predictive studies in models that are non-predictive and Dr. Dean agreed. Dr. Stokes said the concept behind this project is to provide an *in vitro* test that is relatively inexpensive that will estimate relative toxicity and provide some basis for the starting point instead of doing the initial work in animals without any information from *in vitro* studies. Currently the standard procedure is to test a new chemical or product in animals using what the EPA commonly refers to as the six-pack of acute toxicity tests, which includes the acute oral toxicity test. He added that at least this initial *in vitro* test may provide some crude index of relative toxicity that in turn might decrease the number of animals that die or that are needed for each acute toxicity study. The idea is to eventually add the other tests that will make this in vitro toxicity assessment more accurate, and that metabolism has been discussed as the one that should be added next. Dr. Curren provided some information about the rationale for selection of the cell lines being used in the validation study.

Dr. Dean asked why efforts were being directed at trying to validate or find a replacement for LD50 and if the acute toxicity test is of any value. Dr. Stephens pointed out that text on page 21 of the workshop report speaks to the agencies' support for an *in vitro* cytotoxicity test capable of predicting *in vivo* LD50 value because it would reduce animal use. Dr. Willhite referred the SACATM to the executive summary within the workshop report for understanding the recommendations and the goals. He noted that

the workshop attendees recognized the limitations of available systems and the importance of metabolism and used the information available to make recommendations about future directions to advance the effort.

Dr. Acosta said he was unclear about the role of SACATM because the study is already underway. He asked for more background information about the decision-making process for approving this validation study. Dr. Stokes said that the workshop provided many recommendations for research, development, and validation activities, and that an enormous amount of work went into reviewing the current status of available methods to facilitate these expert recommendations. The current study is aimed at carrying out the near term recommendations that could be done quickly, are relatively inexpensive, and would have some benefit for reducing animal use. SACATM could help by providing advice on which of the other recommendations should have the highest priority for further support, especially by Federal agencies. Dr. Portier said he approved funding for this project, which is coming collectively from ECVAM, NIEHS and EPA. He also acknowledged the comments from SACATM and said they would be considered in determining how to continue. He pointed out the important role of SACATM to help filter information from the expert panels and the ICCVAM recommendations to set priorities.

1. Public Comments

Troy Seidle from PETA said he is glad SACATM is now in place. He noted an EPA subcommittee would be looking at alternative strategies for testing within the agency and suggested that group might interface with SACATM. He pointed out that the LD50 is an endpoint commonly required by Federal agencies and internationally and therefore, it is important to develop non-animal testing strategies. He added that although the study being proposed would not answer all questions, it is an important start and he asked the SACATM to support the ICCVAM-ECVAM effort.

Sara Amundson from the Doris Day Animal League reiterated some earlier points and stated that more needs to be done to get industry to incorporate accepted alternatives into their test plans. She urged SACATM to make this a priority.

2. Discussion

Dr. Dean made some general comments. He pointed out that NIEHS primarily appears to be carrying the alternatives program and stressed the importance of involving other agencies. He again invited them to present information about their efforts to SACATM. He also emphasized the importance of collaboration between ECVAM and ICCVAM. In closing, Dr. Dean thanked the SACATM members for their thoughtful discussion.

Dr. Portier also thanked the SACATM for their useful input and patience and Dr. Hartung for his insights on the work of ECAVM. He also thanked Dr. Stokes and his staff for their devotion to NICEATM and ICCVAM activities and the public for their comments.

Dr. Dean adjourned the meeting at 4:12 pm.