

**Department of Health and Human Services
National Institutes of Health
National Institute of Environmental Health Sciences**

**Summary Minutes of the 5th Meeting of the National Toxicology Program Advisory
Committee on Alternative Toxicological Methods**

November 28, 2000

The National Toxicology Program (NTP) meeting of the Advisory Committee on Alternative Toxicological Methods (ACATM) was convened on November 28, 2000 at the National Library of Medicine, National Institutes of Health (NIH) Campus, Bethesda, MD. The meeting was open to the public from 9:00 a.m. to 4:50 p.m. Dr. Kathy Stitzel presided as Chair.

The following ACATM members were in attendance:

- Katherine A. Stitzel, D.V.M. (Chair), Procter & Gamble Company, Cincinnati, Ohio
- Paul T. Bailey, Ph.D., Exxon Mobil Biomedical Sciences, Inc., Annandale, New Jersey
- Michael S. Denison, Ph.D., Professor of Environmental Toxicology, Department of Environmental Toxicology, University of California – Davis, Davis, California
- Elaine Faustman, Ph.D., D.A.B.T., University of Washington, Seattle, Washington
- Alan M. Goldberg, Ph.D., Johns Hopkins University, Baltimore, Maryland
- Sidney Green, Ph.D., Howard University College of Medicine, Washington, D.C.
- A. Wallace Hayes, Ph.D., Gillette Company, Boston, Massachusetts
- Roger McClellan, D.V.M., Consulting Toxicologist, Albuquerque, New Mexico
- Kenneth Ramos, Ph.D., Texas A&M University, Department of Physiology and Pharmacology, College of Veterinary Medicine, College Station, Texas
- Andrew N. Rowan, Ph.D., Senior Vice President, Humane Society of the United States, Gaithersburg, Maryland
- Peter Theran, D.V.M., Massachusetts Society for the Prevention of Cruelty to Animals, Boston, Massachusetts
- Rodger Curren, Ph.D., Institute for *In Vitro* Sciences, Inc., Gaithersburg, Maryland

ad hoc members:

- Betsy Carlton, Ph.D., D.A.B.T., Rhodia, Inc., Raleigh, North Carolina
- Lorraine Twerdok, Ph.D., D.A.B.T., American Petroleum Institute, Washington, D.C.

ICCVAM Agency Representatives:

- William Stokes, D.V.M., ICCVAM Co-Chair, NIEHS, Research Triangle Park, North Carolina
- Richard Hill, M.D., Ph.D., ICCVAM Co-Chair, U.S. Environmental Protection Agency (EPA), Washington, D.C.
- John Bucher, Ph.D., NIEHS, Research Triangle Park, North Carolina
- George Cushmac, Ph.D., U.S. Department of Transportation, Washington, D.C.

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- John Frazier, Ph.D., United States Air Force, Wright-Patterson Air Force Base, Ohio
- Kailash Gupta, Ph.D., Consumer Product Safety Commission, Bethesda, Maryland
- David Hattan, Ph.D., Federal Drug Administration, Washington, D.C.
- Vera Hudson, National Library of Medicine, Bethesda, Maryland
- Abby Jacobs, Ph.D., Food and Drug Administration, Rockville, Maryland
- Margaret Snyder, Ph.D., National Institute of Health, Bethesda, Maryland
- Errol Zeiger, Ph.D., NIEHS, Research Triangle Park, North Carolina
- Harry Salem, Ph.D., Department of Defense, Aberdeen Proving Ground, Maryland
- Marilyn Wind, Ph.D., Consumer Product Safety Commission, Bethesda, Maryland

NICEATM staff:

- Brad Blackard, M.P.H., ILS, Inc., Research Triangle Park, North Carolina
- Loretta Frye, NIEHS, Research Triangle Park, North Carolina
- Debbie McCarley, NIEHS, Research Triangle Park, North Carolina
- Michael Paris, ILS, Inc., Research Triangle Park, North Carolina
- Raymond Tice, Ph.D., ILS, Inc., Research Triangle Park, North Carolina

Other Federal employees:

- Jerrold Heindel, Ph.D., NIEHS, Research Triangle Park, North Carolina
- Carol Robinson, National Heart, Lung, and Blood Institute, Bethesda, Maryland
- Mary Wolfe, Ph.D., Executive Secretary, ACATM, NIEHS, Research Triangle Park, North Carolina

Members of the public:

- Sara Amundson, Doris Day Foundation, Washington, D.C.
- Jessica Sandler, People for the Ethical Treatment of Animals (PETA), Norfolk, Virginia
- Eric Wilson, People for the Ethical Treatment of Animals (PETA), Norfolk, Virginia

Call to Order and Introductions

Dr. Stitzel called the meeting to order at 9:00 a.m., at which time she asked each person in attendance to introduce themselves for the record. Following the introductions, Dr. Mary Wolfe explained to the members of the ACATM the policies and procedures regarding confidentiality and avoidance of conflict of interest situations.

Welcome from the NTP

Dr. Bucher thanked the members of the ACATM for their support, advice, and counsel. He then commented that neither Drs. Olden nor Portier could be present to welcome the ACATM members due to their involvement in a joint U.S./Vietnam meeting in Singapore to discuss research strategies for assessing health effects associated with the use of Agent Orange during the Vietnam War. Dr. Bucher then reviewed briefly two major NIEHS/NTP initiatives of interest to the ACATM.

Dr. Bucher reported that NIEHS has responded to a concern regarding the validation and future regulatory impact of microarray technology, raised by the ACATM during the session on emerging technologies at the March 6-7, 2000 meeting. On December 7th at the National Press Club, the NIEHS will formally announce the creation of the National Center for Toxicogenomics. This Center plans to address the ACATM concern along with other issues in a number of workshops scheduled to be held at academic microarray technology centers and at the NIH. These workshops will include:

- A meeting on functional genomics and environmental health, to be held on December 11, 2000 at the Massachusetts Institute of Technology;
- A satellite meeting to the American Chemical Society Meeting on Human Genomics, on January 27, 2001 at the University of Arizona;
- A meeting on informatics and proteomics, on March 5, 2001 at North Carolina State University; and
- A major meeting on toxicogenomics and issues, including regulatory activities, related to the interpretation of toxicogenomics data, to be held in May 2001 at the NIH campus.

Next, Dr. Bucher discussed the continuing development of transgenic animals for use in cancer assessment and as possible replacement assays for the NTP bioassay. It had been anticipated that ICCVAM would be requested to evaluate the validation status of transgenic cancer assays in the near future. However, at the Workshop on the Evaluation of Alternative Methods for Carcinogenicity Testing (November 1-4, 2000 in Leesburg, VA) sponsored by the International Life Sciences Institute (ILSI) to consider the reliability and performance characteristics of five different transgenic assays using some 20+ different chemicals, it was concluded that these assays would be difficult to validate through the ICCVAM process at this time. Dr. Bucher continued by stating that an ICCVAM evaluation would depend on the scientific community defining exactly the performance characteristics desired for assays of this type, as well as the regulatory expectations for these assays in cancer hazard identification and risk assessment.

Dr. Faustman asked about the process for defining the regulatory context. Dr. Bucher replied that the pharmaceutical industry and the Center for Drug Evaluation and Research (CDER) at the Food and Drug Administration (FDA) have been interpreting the results of transgenic assays in terms of a weight-of-evidence approach for hazard identification. However, the current database is far too limited for Federal regulatory agencies to decide how transgenic assay results would be used for quantitative risk assessment. NIEHS wants to use the results from transgenic studies to identify carcinogens, but is not yet fully comfortable in using such data as the sole criteria, especially when assay results are negative. There is hope that the results from selected transgenic studies in conjunction with data from toxicological, structure-activity, and class studies would be sufficient.

Dr. Curren asked if the ILSI data were available. Dr. Bucher replied that the data from the ILSI initiative are available in summary format, but that the complete database was still being compiled. He encouraged members of the ACATM to contact Dr. Denise Robinson at ILSI for additional information on the ILSI transgenic study results. Dr. Hayes asked if ILSI could summarize their findings at the next ACATM meeting. Dr. Bucher stated that he could not commit to a presentation for ILSI, but that Dr. Robinson could be asked to give such a talk.

Dr. Hayes asked about the status of the ICCVAM Authorization Bill of 2000. Dr. Stokes replied that it had passed in the House of Representatives and has been referred to the Senate for consideration. Dr. Hayes asked what would happen if the bill failed to be authorized this year. Dr. Stitzel replied that the authorization process would be started again next year.

Dr. Bucher completed his remarks by presenting certificates and letters of appreciation on behalf of NIEHS/NTP to the two retiring ACATM members—Drs. Elaine Faustman and Roger McClellan. He thanked them for their commitment and service, as did Dr. Stitzel and other members of the ACATM.

Update on NICEATM and ICCVAM Activities

Dr. Stokes reviewed the activities of ICCVAM and NICEATM during the six-month period since the last ACATM meeting. He first described the status of the two test methods previously evaluated by ICCVAM—the Murine Local Lymph Node Assay (LLNA) and Corrositex®. The LLNA, the first test method peer review coordinated by ICCVAM, was evaluated by a Peer Review Panel in September 1998. The Panel concluded that the LLNA was a valid substitute for the guinea pig test for assessing allergic contact dermatitis potential of chemicals. The results of this evaluation and ICCVAM recommendations were forwarded to Federal agencies and regulatory acceptance announced in October 1999. Currently, relevant agency guidelines are being revised; written notification will be issued to the regulated community upon their completion. A draft test guideline is in preparation and will be circulated in the near future for adoption by the Organization for Economic Cooperation and Development (OECD) at the international level. He noted that Dr. Hattan, co-chair of the ICCVAM Immunotoxicology Working Group (IWG), would provide an update on a LLNA Training Workshop scheduled for January 25-26, 2001.

Corrositex® was the second test method to be evaluated by ICCVAM. At its January 1999 Meeting, the Peer Review Panel concluded that this method could be used in a tiered testing strategy for evaluating corrosivity. The conclusions of the Panel were endorsed by the Corrosivity Working Group (CWG) and ICCVAM and forwarded to Federal agencies. Regulatory acceptance was announced by most of the regulatory agencies in late 1999 and by the U.S. Environmental Protection Agency (EPA) in the spring of 2000. NICEATM is preparing a draft OECD test guideline to achieve adoption and use of the method at the international level.

Dr. Stokes then reviewed the three meetings organized by ICCVAM during the last six months. These included an expert panel meeting on the Frog Embryo Teratogenesis Assay—*Xenopus* (FETAX), an independent peer review of the Up-and-Down Procedure (UDP) for acute oral toxicity, and an international workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity.

The FETAX expert panel meeting was held May 16-18, 2000. Dr. Stokes detailed the events leading to the expert panel meeting. He stated that in May 1998 the U.S. EPA Office of Research and Development asked ICCVAM to evaluate the validation status of FETAX for its usefulness in generating data for regulatory purposes and identify additional research and

development that might advance the usefulness of this method. An ICCVAM Developmental Toxicity Working Group (DTWG) was established to work with NICEATM to arrange the expert panel meeting. NICEATM conducted a review of published and unpublished FETAX data and prepared a draft comprehensive background review document (BRD) on FETAX in 1999. An *ad hoc* group from NIEHS assisted in compiling and analyzing the available data. A Federal Register (FR) notice was issued seeking names of experts to serve on the panel, announcing the availability of the BRD, and providing information on the public meeting. The DTWG developed questions for the expert panel to address in their evaluation and recommended 43 expert scientists for the panel. The experts were divided into five breakout groups—protocol, reliability, performance, environmental applications, and research and development. Dr. Stokes listed the members of each breakout group and described the agenda for the two-and-a-half-day meeting chaired by Drs. George Daston and Elaine Faustman. The objectives of the meeting were to develop a consensus on the current validation status of FETAX; develop consensus on its current and potential usefulness for specific purposes; identify research and method development efforts that might improve the accuracy and reproducibility of FETAX, and to identify validation studies that would further characterize the usefulness and limitations of FETAX. The final report is in preparation and should be released early in 2001. Dr. Stokes stated that Dr. Faustman would present the findings of the FETAX Expert Panel later in the meeting.

Dr. Curren asked if additional FETAX data had been received in response to the FR notices. Dr. Stokes replied that a limited amount of unpublished data from industry were received, but that the data could not be used in a formal assessment of FETAX due to the lack of specific information on the chemicals tested.

Dr. Stokes next reviewed the ICCVAM evaluation of the UDP for acute oral toxicity. The UDP was proposed to the OECD by the U.S. EPA in 1996 and adopted as OECD Test Guideline (TG) 425 in 1998. Later in 1998, the OECD adopted a harmonized integrated hazard classification system that necessitated changes to the UDP Test Guideline. In 1999, the United States assumed responsibility for drafting a revised UDP that incorporated appropriate changes. A U.S. EPA task force, headed by Dr. Amy Rispin was established to conduct the necessary work. In 1999, the U.S. EPA requested that ICCVAM assess the validity of the revised UDP, that included a revised Primary Test, a revised Limit Test, and a Supplemental Test for determining slope and confidence interval. ICCVAM established an Acute Toxicity Working Group (ATWG) to work with NICEATM to organize the independent peer review panel evaluation. NICEATM worked with the Task Force to prepare a BRD. In June 2000, a FR notice announced the availability of the UDP BRD, requested comments on the BRD, and announced the July 25th Peer Review Meeting. Dr. Stokes listed the members of the Peer Review Panel, which was co-chaired by Drs. Kurt Klaassen and Diane Gerkin. The Peer Review Panel was divided into four sections, with one group for each of four major review topics: protocol considerations, revised UDP Primary Test, revised UDP Limit Test, and UDP Supplemental Test. The evaluation guidance to the Panel asked: (1) whether the revised UDP was evaluated sufficiently and its performance satisfactory to support its adoption as a substitute for the currently accepted UDP and as a substitute for the conventional LD50 test for acute oral toxicity (EPA OPPTS 870.1100); and (2) with respect to animal welfare, did the revised UDP adequately consider and incorporate, where scientifically feasible, procedures that refine, reduce, and/or replace animal use. The conclusions and recommendations of the Panel were sent with a U.S. delegation for

consideration at an OECD expert meeting on this topic in Paris, France in August 2000. Issuance of a final report is pending the development of a confidence interval calculation procedure to be incorporated into the Primary Test Method. Once finalized, the confidence interval proposal will be circulated to the UDP Panel for comments and public comments requested via a FR notice. Comments received will be summarized in the UDP final report that will be forwarded to Federal agencies and made available to the public.

Dr. Hayes asked if the confidence interval procedure would be peer reviewed. Dr. Stokes replied affirmatively, stating that the proposal would be forwarded to the Panel for peer review via a public teleconference meeting early in 2001. Dr. Stokes added that the procedure is simply a calculation based on data from the Primary Test and did not require the use of any additional animals.

Dr. Stokes next discussed the third major activity conducted since the last meeting of the ACATM, which was a four-day International Workshop on *In Vitro* Methods For Assessing Acute Systemic Toxicity held on October 17-20 in Arlington, Virginia. ICCVAM received a request from EPA and several hundred letters from members of the public requesting review of the Multicenter Evaluation of *In Vitro* Cytotoxicity (MEIC) proposed test battery of three *in vitro* methods to estimate acute toxicity in humans. NICEATM subsequently prepared a summary analysis of the 10-year MEIC effort, which evaluated 50 chemicals and 65 different test methods. After consideration of this analysis, ICCVAM recommended that an expert workshop should be convened. A workshop organizing committee co-chaired by Drs. John Frazier and Phil Sayre was established to work with NICEATM to organize the workshop. ICCVAM issued FR notices announcing the workshop, requesting data on relevant *in vitro* methods, seeking nominations for expert scientists to participate in the workshop, and indicating the availability of background materials. Dr. Stokes presented a list of the organizing committee, the invited experts, and the ICCVAM participants, and then described the organization of the workshop. Breakout groups were assembled to review: (1) *in vitro* methods for acute toxicity; (2) *in vitro* methods for assessing toxicokinetic parameters; (3) *in vitro* methods for assessing organ-specific toxicity; (4) chemical data sets that could be used to validate these *in vitro* methods. The workshop breakout chairs are preparing written reports and a complete workshop report will be available in 2001.

Dr. Stokes next discussed future ICCVAM/NICEATM activities. These included:

- Evaluation of *in vitro* screening methods for endocrine disruptors. At the request of and with support from the U.S. EPA, NICEATM will prepare a BRD on *in vitro* screening methods for endocrine disruptors and will organize an ICCVAM independent peer review of these methods in late 2001 or early 2002. The *in vitro* assays under consideration include receptor binding and transcriptional activation assays for androgens and estrogens.
- Evaluation of the human corneal epithelial (transfected), transepithelial permeability assay (HCE-T TEP), an *in vitro* alternative test method sponsored by The Gillette Company for assessing the ocular irritancy of surfactants and surfactant-containing formulations. An ocular toxicity working group (OTWG) examined a pre-validation submission and provided comments to the sponsor. The validation study is in progress and a formal submission is expected in early to mid 2001.

- Evaluation of EpiOcular™, a second *in vitro* alternative test method for assessing the ocular toxicity of surfactants and surfactant-containing formulations. This assay was submitted for consideration by Colgate-Palmolive in cooperation with the Institute for *In Vitro* Sciences, Inc.
- Three *in vitro* test methods that have been validated by the European Centre for the Validation of Alternative Methods (ECVAM). These are the rat skin transcutaneous electrical resistance (TER) assay for assessing dermal corrosivity, EpiSkin™ for assessing dermal corrosivity, and the 3T3 neutral red uptake (NRU) assay for assessing phototoxicity. Dr. Stokes stated that after his presentation, Dr. Hill would discuss an ICCVAM proposal for reviewing ECVAM-approved methods.

Dr. Stokes discussed the March 2000, ACATM recommendation that all endocrine disruptor methods should be evaluated by ICCVAM, not just the *in vitro* methods. ICCVAM discussed the ACATM recommendation, and determined that the test method review capacity of ICCVAM and NICEATM was too limited, based on current and projected resources, to review all the proposed endocrine disruptor methods. ICCVAM also noted that an ICCVAM review was not necessarily appropriate for all new methods, especially if the test method was agency-specific. ICCVAM, however, felt that if the agencies do use an ICCVAM-like process, they should include critical features of the ICCVAM test method review process. ICCVAM also considered the proposed U.S. EPA process for endocrine disruptor test method evaluations and concluded that it appeared to be an acceptable means of evaluating the validation status of proposed methods.

Dr. Stokes concluded his presentation by acknowledging the contributions of ICCVAM agency representatives, the agency staff that have served on interagency ICCVAM working groups, and the NICEATM staff. The ACATM members requested that the outstanding effort of the NICEATM staff be formally acknowledged in the ACATM record.

Dr. Rowan asked if ICCVAM had concluded that the endocrine disruptor arena is only of interest to the U.S. EPA. Dr. Stokes replied by saying that there has been considerable interest by other Federal agencies. Dr. Hayes made a motion that all endocrine disruptor methods should be validated by ICCVAM. Dr. McClellan seconded Dr. Hayes' motion by expressing satisfaction with the ICCVAM process and stating that developing validated testing methods to detect endocrine disruptor effects is an issue of broad national and international concern, which mandates the use of well-validated evaluation methodology. He emphasized that one of the strengths of the ICCVAM process is that thus far it has been largely apolitical. He noted that activities focused in one agency often become much more political and that political considerations have already impacted the revision of cancer risk assessment guidelines at EPA in terms of significant delays. He expressed concern that if the endocrine disruptor methods were not reviewed by ICCVAM, that similar adverse impact on the program might occur. He emphasized that ICCVAM already has an efficient, effective structure in place, and in fact, the resource requirements for review by ICCVAM might very likely be less than an EPA review. Thus, the cost of having ICCVAM conduct the reviews is truly not an issue, since funds designated for this purpose could simply be transferred by EPA through an interagency agreement. He felt that they have the primary responsibility for providing funds to ICCVAM to support its mandate. He urged that a critical review be made of the resource requirements and

projected time schedule necessary to carry out these reviews. He stated that the review of *in vitro* methods is a good start, but that the ICCVAM should review both the *in vitro* and the *in vivo* methods.

Dr. Hayes asked for clarification regarding why EPA rejected having ICCVAM review all endocrine disruptor testing methods. Dr. Stokes replied that the U.S. EPA had requested that ICCVAM review the *in vitro* endocrine disruptor methods in part because they felt sufficient data were available for their review. The *in vivo* methods still need additional validation studies conducted and will not be ready for peer review until much later. Also, the U.S. EPA feels that since they were given the statutory mandate from Congress to develop and validate an endocrine disruptor screening and testing program, they should therefore assume the responsibility for the peer review of these methods. Dr. Hill agreed, stating that the ICCVAM process is time consuming and that it would be better to have ICCVAM evaluate the *in vitro* methods while the U.S. EPA proceeds with the *in vivo* methods, especially in light of the limited resources for ICCVAM evaluations. He also stated that OECD is coordinating validation of some of the *in vivo* methods.

Dr. Green endorsed Dr. McClellan's suggestion for a review of resources necessary for the endocrine disruptor methods. He expressed concern as to the rapidity at which ICCVAM has been accepting methods and agreed that ICCVAM should not evaluate all endocrine disruptor test methods. He cautioned that a significant increase in workload might potentially compromise the quality of ICCVAM's work and that reviewing all endocrine disruptor methods might detract from the review of other alternative methods. Dr. Stokes replied that additional resources would be necessary if ICCVAM were tasked to evaluate additional methods. Dr. Bucher commented that the U.S. EPA has been very cooperative and that there have been discussions about approaches for combining the time for ICCVAM activities with shared memberships and U.S. EPA involvement. He noted also that the *in vitro* methods have the highest chance of reducing animal use. Dr. Goldberg asked about the criteria and the processes used by ICCVAM in determining the next set of assays to evaluate. Dr. Stokes reviewed the current ICCVAM procedures for that process.

Dr. McClellan commented that ICCVAM has developed enough experience to determine the strategic orientation and the decision criteria that should be used for the deployment of resources. Dr. Faustman endorsed a 5- to 10-year strategic plan and suggested that the most appropriate assays (i.e., *in vitro* ones) might be reviewed preferentially if a plan existed. She commented also that there should be no appearance of a double standard in regard to assay validation and that there is a need to assess the strategic nature of each assay. Dr. Curren agreed with Dr. Faustman and asked Dr. Stokes about the ACATM motion from the March 7-8, 2000 meeting regarding the ICCVAM testing of all endocrine disruptor assays. Dr. Stokes indicated that the response from EPA was that ICCVAM would only evaluate specific *in vitro* assays. Dr. Rowan suggested that ICCVAM is evolving from its original premise, while Dr. Goldberg stated that there was a need to "step back" and be strategic and to optimize the use of the available resources. Dr. Stitzel commented that the issue of distinction between *in vitro* and *in vivo* testing concerning endocrine disruptors should be resolved. Dr. Hayes agreed with Dr. Stitzel and the discussion continued concerning the resources of the U.S. EPA and how those resources apply to the endocrine disruptor program. A suggestion was made that there could be

a joint venture where the U.S. EPA conducts the studies and then submits the data to ICCVAM for review.

Dr. Stitzel said that the ACATM would continue this discussion after lunch to provide an opportunity for the appropriate motions to be drafted.

ICCVAM Procedures for Test Methods Endorsed by ECVAM

Dr. Hill started his presentation by discussing briefly how the different Federal agencies interact with international organizations such as the OECD. He then focused on the types of interactions that occur between ICCVAM and ECVAM, suggesting that increased harmonization between the two organizations should be encouraged. Dr. Hill discussed ECVAM's involvement in all aspects of the validation process and its interaction with the European Commission, European Union, and OECD. Dr. Hill then identified the two major differences between the process used by ECVAM and the one used by ICCVAM. First, ICCVAM has not gotten involved in conducting validation studies, and secondly, all aspects of the evaluation process are public. To decrease the time needed to evaluate and potentially accept new alternative assays, Dr. Hill proposed that ICCVAM develop an expedited review process for assays already validated by ECVAM. In this process, NICEATM would review the validation studies and the peer-reviewed report and subsequently assess them for completeness using the ICCVAM submission guidelines and criteria for assessing validation. If no major problems are identified, then ICCVAM would develop a draft position on the method, to be published in the Federal Register for comment. The resulting comments would be addressed, and if no major problems are identified, ICCVAM would make recommendations to the Federal agencies and inform ECVAM. If major issues are identified, a complete assessment of the test method would be conducted utilizing the ICCVAM peer review process and criteria.

During the resulting discussion, the ACATM raised concern about situations where ECVAM might differ qualitatively and quantitatively from ICCVAM in the evaluation process (e.g., in the criteria for accepting FETAX), and questioned the length of time that would be required for this expedited process. Dr. Hill replied that the expedited process might take about half the time as the regular evaluation process. Dr. Rowan asked about what constituted a major issue and how data, especially proprietary data, could be obtained to support the initial evaluation. Dr. Hill replied that in the past, data have come forth readily and that the Federal government does not have a problem with proprietary data. Dr. Green also asked about the identity of the types of major problems anticipated; Dr. Hill replied that ICCVAM has not yet identified any major issues in ECVAM-validated alternative assays (e.g., dermal corrosivity). Drs. Faustman and Hill discussed what issues in an assay might be deemed major and minor in regards to ICCVAM and ECVAM criteria. It was suggested that these should be defined. The discussion among the ACATM members continued, with a suggestion that ECVAM and ICCVAM should notify each other when a validation study is initiated. The ACATM suggested also that the criteria for starting the process of an ICCVAM review of an ECVAM-validated method should be clearly defined.

Implementation of ICCVAM Recommended Methods

Dr. Hattan discussed the LLNA training workshop scheduled for January 25-26, 2001 at the NIH Natcher Center. The purpose of the workshop is to describe the technical aspects of the LLNA, its advantages and limitations, and its appropriate uses and interpretations to interested scientists in regulatory agencies, contract laboratories, and industry. Dr. Stokes commented that the workshop was a cooperative effort between ICCVAM and ILSI.

Report on the Peer Review of the Up-and-Down Procedure (UDP)

Dr. Stitzel reviewed the UDP protocol. The Primary Test is based on a staircase design (i.e., dosing single animals in sequence rather than groups of animals) and a set of stopping rules is used to determine if enough data are collected to determine the LD50. The calculations are based on the maximum likelihood method, the slope is assumed and not calculated, and the calculations can be conducted using commercially available software. In conducting the UDP, the initial dose should be based on all available information, the most sensitive sex should be used, the dose progression can be adapted to meet the needs of the test substance, and the observation period between animals can be increased. The UDP also includes a Limit Test of 2000 or 5000 mg/kg that uses up to five animals; the test is completed once three of the five animals have survived or have died. If three animals survive, then the LD50 is greater than the limit dose. If three animals die, the LD50 is lower than the limit dose. Dr. Stitzel stated that the strengths of the method include the use of a reduced number of animals, a point estimate of the LD50, all classification systems are met, death is used as an endpoint, and similar observations as used in TG 401 are made. The weaknesses of the method are that the slope is assumed and not calculated, females only are currently recommended (because in most cases females are more sensitive than males), it is not suitable for a test substance with highly delayed toxicity or for inhalation studies, and the increased study duration and thus cost and complexity.

Recommendations and Conclusions of the UDP Peer Review Panel

Dr. Hayes presented the Panel conclusions concerning the Primary Test, the Limit Test, and the Supplemental Test. The performance of the Primary Test is satisfactory and generally exceeds the performance of TG 401. The test method provides, with fewer animals, both an improved estimate of the LD50 for the purpose of hazard identification and the potential for better overall information on acute toxicity. Compared to TG 401, the main disadvantages are that it will take longer to conduct each study, the cost of each study will be increased, and the study protocol is more complex. The Panel recommended adoption of this test method. The Panel concluded that the Limit Test for 2000 or 5000 mg/kg is expected to perform as well as or better than the current TG 401 limit test, with a reduction in the number of animals needed to conduct the test. The Panel recommended adoption of this test also. In contrast, due to insufficient information and justification, the Supplemental Test for slope and confidence interval was not evaluated and was not recommended for adoption.

Dr. Hayes then discussed the general and specific recommendations of the Panel for the two test methods recommended for adoption. The recommendations included the use of either constant volume or constant concentration of the test material, the use of either sex unless information

suggests one sex more sensitive, no reference to littermates, the use of 8- to 12-week old animals, and that individual body weights on the day of dosing must be within 20% of mean body weight for all animals dosed. The Panel also made recommendations concerning the structure and format of the guidelines. These suggestions included reorganization to improve clarity, the need for additional guidance on the transition from the Limit to the Primary Test, the starting rules, obtaining and using all pre-start information, and increased emphasis on the usefulness of the information gained beyond the LD50. They recommended also that a comprehensive validated software package, including data sets for in-house validation for compliance with Good Laboratory Practice (GLP) guidelines should be included, as well as additional justification for the default starting dose of 175 mg/kg. For the Supplemental Test, Dr. Hayes indicated that the purpose of the utilization of the slope and the confidence interval in human environmental risk assessment should be stated and consideration should be given as to whether slope and CI are the most appropriate parameters for risk assessment, or whether risk assessment needs to be addressed more directly. Dr. Hayes concluded by stating that the two recommended test methods did provide for reduction and refinement but not replacement of animal use.

Dr. Bailey asked about the cost of each test; Dr. Stitzel replied that there were no firm numbers but that increased cost compared to TG 401 might be anticipated. Dr. Rowan asked if the UDP was better than TG 401. Dr. Stitzel responded by saying that it was better and more accurate according to computer simulations. Dr. Goldberg commented that it would be useful if the Bureau of National Affairs (BNA) were notified of the inaccuracies in its article on the UDP Peer Review and asked to print a revision.

Dr. Stitzel provided an update on proceedings since the writing of the revised OECD UDP Guideline. She explained that the OECD decided to retain the language about the preferred use of females. However, the UDP Panel's recommendation on using constant concentration as well as constant volume during test substance administration resulted in the OECD recommending the use of either approach in all three acute toxicity test guidelines. Also, the Panel recommendation concerning the 20% weight change guidance will be used in all three guidelines. The decision made at the OECD meeting of all country coordinators was that the three revised tests would be accepted and TG 401 deleted in the very near future.

Dr. Stitzel then asked for public comment.

Public Comment

Ms. Jessica Sandler (PETA) asked if the confidence interval proposal would delay the OECD timeline for deleting TG 401. Dr. Stitzel was not sure if the timeline would be affected.

Ms. Sandler asked also if the United States was only one of two countries that still required a limit dose of 5000 mg/kg in acute toxicity testing; Dr. Hayes replied he did not know.

Ms. Sandler commented that she was surprised that data were not available to support the claim that the use of the UDP would result in a reduction in animal use. Dr. Stitzel responded by saying that while the statistical simulations indicate a reduction in animal use, no experimental data have been generated to support the simulations.

Ms. Sandler continued by expressing her concern and the concern of multiple animal protection organizations about the OECD and the U.S. EPA timeline for deleting TG 401, the Department of Transportation's continued use of animals instead of Corrositex® for assessing corrosivity, and the scientific validity of the endocrine disruptor program. She continued with concerns regarding the delays in the utilization of ICCVAM-endorsed methods, particularly at the OECD level. Further, she commented about concerns pertaining to the validation criteria of endocrine disruptor methods using animals, especially in light of ECVAM withdrawing from such studies. Ms. Sandler commented also that, in contrast to ICCVAM, she did not believe that the U.S. EPA Scientific Advisory Panel represented an independent scientific review panel. She also suggested more rapid interaction between ICCVAM and ECVAM. Ms. Sandler completed her comments by asking ICCVAM to increase its efforts in evaluating alternative test methods.

Next, Ms. Sarah Amundson (Doris Day Animal League) stated her support of HR-4281 (the ICCVAM Authorization Bill), noting that there is both Republican and Clinton administration support for its passing and that both alternatives and animal test methods are referenced as the purview of ICCVAM. Ms. Amundson commented that the recent California law, signed in September 2000, mandates that any ICCVAM recommendation that is adopted by the relevant Federal agency is a test method that must be used by industry in the state of California. She recommended that the agency questionnaires in the original ICCVAM report (1997) be updated. Ms. Amundson concluded by stating stakeholder education is needed about ICCVAM.

Dr. Stitzel concluded the morning session at 12:20 p.m.

Afternoon Session

Dr. Stitzel called the afternoon session to order at 1:15 p.m. The first topic of concern was the proposed ACATM motion on endocrine disruptors. After extensive discussion the following motion was proposed by Dr. Hayes and seconded by Dr. Goldberg:

ACATM Motion on Endocrine Disruptors:

- *The ACATM expresses grave concern at the bifurcated approach being taken with review of methods for evaluation of endocrine disruption activity, with ICCVAM considering in vitro methods and with the U.S. EPA proposing to review in vivo methods using an ICCVAM-like approach. The Committee's primary concern is that both in vitro and in vivo methods be subjected to the same rigorous peer review and validation process to ensure the highest likelihood of acceptance by the regulatory agencies, the scientific community, and the public.*
- *The ACATM Committee recommends that ICCVAM and the U.S. EPA work together to identify the resources and the related time schedule needed for ICCVAM to evaluate both in vitro and in vivo endocrine disruptor methods.*
- *The Committee urges that, with this information in hand, the appropriate senior management consider whether it would be in the best national interest for ICCVAM to evaluate the validation status of both the in vitro and in vivo methods rather than using the bifurcated approach proposed currently.*
- *Resources should be made available from the U.S. EPA, ICCVAM, and other parties as appropriate.*

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The ACATM voted unanimously to accept the motion (12 yes including the Chair, 0 no, 0 abstentions). The ACATM requested that this motion be forwarded through NTP to the U.S. EPA; Dr. Stitzel agreed to prepare an accompanying cover letter.

The next topic of discussion was the proposed ACATM motion on strategic planning.

The ACATM requested information on how ICCVAM decides what methods to pursue and what criteria are followed. The ACATM believes a strategic plan would facilitate such decisions and selection criteria for methods are needed. The NTP acknowledged that it is currently working on a strategic plan that would be brought to ACATM for review and input. Currently NICEATM pre-screens test method submissions for completeness and forwards those that are sufficiently complete to ICCVAM for consideration. Thus far there has not been an overabundance of submissions.

The following motion was moved by Dr. Hayes and seconded by Dr. Goldberg:

ACATM Motion on Strategic Planning:

- *The Committee again expresses its pleasure with the progress being made by ICCVAM and encourages further planning to maximize continuing success.*
- *The Committee recommends that ICCVAM and its member agencies and representatives initiate a strategic planning process.*
- *This process should include projecting major areas of activity for ICCVAM, the decision criteria to be used in prioritizing those activities, and soliciting and deploying resources.*
- *The Committee stands ready to assist in reviewing the strategic plan and in providing feedback to ICCVAM and its member agencies.*

The members unanimously accepted the motions (12 yes including the Chair, 0 no, 0 abstentions).

Development and Validation of New Test Methods

FETAX Expert Panel Meeting

Dr. Tice reviewed the mechanistic basis for FETAX and summarized the available chemical and environmental databases. The former included 137 single substances tested in 276 studies, while the latter included 124 environmental samples (water, sediments, soil) evaluated in ten studies. He then outlined the standard FETAX protocol, including range-finding and definitive tests, the use of positive controls, and the various single and multiple decision criteria used for identifying a positive response. Dr. Tice summarized the methods used to assess the performance characteristics of FETAX for identifying mammalian (experimental laboratory animal and human) teratogens and described the five validation studies conducted to evaluate test method reliability.

FETAX Panel Conclusions and Recommendations

Dr. Faustman described the division of the expert panel into five breakout groups—protocol, reliability, performance, environmental applications, and research and development. She then summarized the conclusions and recommendations of each breakout group.

The Protocol Breakout Group recommended protocol changes in five general areas. In terms of animal husbandry, the Group recommended more quality control in regard to feed, housing, breeding conditions/age, and the commercial sources of *Xenopus*. For training and communications, they recommended an annual user's workshop, in-laboratory training at expert laboratories, and expansion/clarification of the Atlas of Abnormalities. For experimental design, they recommended a quality control review/peer review of identified malformations, an increase in the number of breeding pairs, the tracking of individual embryos, and efforts to define optimal exposure volumes/embryo loadings. For endpoints, they recommended that if FETAX were to be used for an evaluation of developmental toxicity, endpoints such as embryo length, malformations, functional deficits, lethality, and developmental stage should be evaluated in a manner more consistent with those required in the current regulatory context. For data analysis, they recommended the following: statistical analysis be based on the individual embryo; estimates of the effective concentration inducing malformations in 50% of the embryos (i.e., the EC50) and the concentration inducing lethality in 50% of the embryos (i.e., the LC50) be based on statistically significant increases in malformations or embryo lethality, respectively; the teratogenic index (TI) and the minimum concentration to inhibit growth (MCIG) should be reconsidered as measures of teratogenicity; the formal statistical comparison of slopes should be required; the use of Student's t-test should be appropriate; data should not be extrapolated beyond the range of observed data; and statistics should be used in the overall decision-making process.

The Reliability Breakout Group concluded that the FETAX results were excessively variable (both within/between laboratories), that variability was observed for all major measures, and that the excess variability casts doubt on the credibility and usefulness of assay. Therefore, they concluded that FETAX should not be used for regulatory decision-making at this time. The Group recommended the following: subsequent to significant protocol modifications, FETAX reliability would need to be re-evaluated in blinded tests; reassessment would need to use coded compounds representing a large number of chemical and mechanistic classes from which a range of toxic outcomes would be expected, and the size and scale of this validation should be based on scientific rationale for the number and nature of test compounds and for the number of laboratories conducting comparable studies.

The Performance Breakout Group concluded also that FETAX had no current role in regulatory human health risk assessment, but that potential uses may include non-regulatory environmental screening and prioritization. They expressed special concern about the adequacy of the *in vivo* data used to judge the performance of FETAX and recommended that a special working group be established to develop a mammalian teratogen database suitable for use in the development of alternative test methods. The Environmental Breakout Group concluded that the role of FETAX in ecological risk assessment could not be addressed adequately due to the limited information provided. They recommended that more information be collected on assay performance in

ecological assessments, the toxicology of *Xenopus*, and the sensitivity and utility of *X. laevis* compared to other amphibian species (e.g., Native North American species, diploid *X. tropicalis*).

For optimization of FETAX, the Research and Development Breakout Group recommended the following: a critical assessment of the organism (*X. laevis* and *X. tropicalis*); a consistent, standardized, reliable source and supply of animals; refinement/standardization of FETAX maintenance conditions; additional effort to optimize the microsomal activation system; rigorous, intensive and standardized technical training; the inclusion of additional assay endpoints; characterization of all malformations; and an easily accessible database of FETAX results and malformations. Possible assay advancements considered included the use of transgenic animals to evaluate gene expression and extension to other specific assays (e.g., limb development, tail resorption, reproductive toxicology).

The resulting discussion focused on the process for eliminating an assay from peer review and the methods used to identify all information that should be considered when a method is nominated for review.

Report on the International Workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity

Background and Objectives of the *In Vitro* Workshop

Dr. Sayre, co-chair of the Organizing Committee and the Workshop, reviewed briefly the background to the workshop, including the regulatory need for acute toxicity data, and listed the members of the organizing committee. He next stated the objectives of the workshop, which included:

- reviewing the status of various *in vitro* methods for assessing acute systemic toxicity, including screening (e.g., MEIC), toxicokinetic parameters, and specific organ toxicity;
- recommending methods for prevalidation/validation;
- recommending validation study designs;
- identifying reference chemicals for validation studies; and
- identifying priority research efforts to support *in vitro* methods (e.g., microarrays).

Dr. Sayre described the structure of the workshop and the four breakout groups—screening methods, toxicokinetics, organ specific toxicity, and chemical data sets. Dr. Sayre concluded by stating that the four breakout groups worked together to answer the questions developed by the Organizing Committee and to make recommendations and conclusions.

Screening Methods Breakout Group

Dr. Curren represented the Screening Methods Breakout Group. He listed its members, re-stated the objectives as described by Dr. Sayre, and summarized the group's conclusions and recommendations. The Group concluded that it is biologically plausible that cell death *in vitro* could be used to predict the acute LD50 *in vivo*. The short-term goal of the Group was to identify methods for reducing animal use in acute toxicity studies; the long-term goal was to

replace animal tests. Agency representatives at the workshop agreed with the Group members that if a validated *in vitro* cytotoxicity test were developed for predicting the approximate rodent LD50 value *in vivo*, then there would be a significant reduction in animal use. The Group discussed a number of specific parameters about the protocols such as the most appropriate cell lines to use, optimal exposure durations, and the most appropriate endpoints to assess and how they should be measured. The Group recommended developing an assay for estimating the starting dose for current *in vivo* acute toxicity test methods, but to focus preferentially on one that may eventually be able to predict an LD50. At least two cell lines (at least one rodent and one human cell line) should be examined in pre-validation and validation studies; the cell lines should be normal and not transformed. The Group recommended that a working group be established to define the specific test protocols to be included in a prevalidation study, taking into account the considerations regarding cell types, exposure times and endpoint measurements, and providing adequate justification for choices made with reference to the existing scientific literature.

The Group concluded also that quantitative structure activity relationship (QSAR) technology could add to the approach and be instrumental in developing the appropriate test chemicals for validation studies. They recommended that a review be conducted concerning the possible role of QSAR in assessing acute toxicity. In the immediate future, simple predictive systems should be examined for information that could be considered together with a basic cytotoxicity number to estimate gut absorption, blood-brain barrier passage, and kinetics *in vivo*. Longer-term research and development would focus primarily on human systems and trying to develop simple predictive models for human acute toxicity. Genomics and proteomics may provide long-term solutions.

Dr. Curren concluded his comments by stating that a guidance document would be produced that will explain the approach to use and other details for an *in vitro* study. Over a two to three-year period, pre-validation studies could be designed and move into full validation studies. Eventually, an approximation of the LD50 may be possible using *in vitro*-based assays only.

Dr. Hayes commented that it would be difficult to meet the two-to-three month deadline for the development of a guidance document and the two-to-three year deadline for completion of the validation studies. He suggested not losing sight of practicality.

Toxicokinetics Breakout Group

Dr. Frazier reviewed the deliberations of the Toxicokinetics Breakout Group. The challenge to this Group was to evaluate the capabilities of *in vitro* methods for providing toxicokinetic information (absorption, distribution, metabolism, and elimination) that could be used to estimate target organ dosimetry for acute toxicity testing and to provide recommendations for future research needs to accomplish this goal. The role of QSAR in toxicokinetic determinations was also to be explored. A challenge raised during the workshop was to determine whether information about chemical kinetics in biological systems would be expected to improve the correlation between *in vitro* toxicity test results and acute *in vivo* toxicity. Recommendations of this Group included the need to conduct a critical evaluation of the outliers in the predictive relationship between *in vitro* effective concentration and the *in vivo* lethal concentrations, while

determining if kinetic factors make a significant contribution. Major kinetic factors that affect acute toxicity that are not evaluated by *in vitro* cytotoxicity tests include bioavailability, clearance (metabolism, renal, biliary, exhalation), and protein binding.

Dr. Frazier discussed two major approaches to QSARs. One is a purely correlative type of an approach, while the other is knowledge based. The correlative approaches, in terms of kinetics, have mostly been related to issues about determining various physical and chemical parameters. Software is available to estimate physical properties. Physical chemical characteristics of the test material should be known early in the study. There are several knowledge-based systems that can predict metabolism qualitatively. They provide some guidance about what may be potential metabolites that need to be considered. The Group concluded that QSAR was not ready to significantly assist at this point.

Dr. Frazier continued by stating that the real issue for the *in vitro* approach is the need to consider the role of metabolism in generating the ultimate chemical species of concern for toxicology. Also, the actual concentration of the chemical experienced by the target cell is important in extrapolating *in vitro* test results to the *in vivo* situation. Another concern is whether regulatory agencies were going to continue to base acute toxicity regulatory policy on animal data, or base it on human toxicity estimation. If it is based on human toxicology, then a very strong human-oriented program is needed. Dr. Frazier concluded by stating that the human hepatocyte system would most likely provide the most information in regard to the issue of metabolism. However, considerable effort is needed to identify and minimize sources of variability (e.g., culture conditions).

Organ Specific Toxicity Breakout Group

Dr. Stitzel first presented a list of the members of the Organ Specific Toxicity Breakout Group. The charge to the Group was to review *in vitro* methods that predicted specific organ toxicity and toxicity associated with specific settings or organ functions. The Group recommended identifying the organs that are most likely to be affected by a 24-hour exposure, the types of toxic effects that would be linked to each organ system, and the types of toxic effects that could be detected using an *in vitro* assay. The organs that would be affected by acute studies include cardiovascular, circulatory, respiratory, skin, liver, kidney and brain (including blood brain barrier). Dr. Stitzel said that, as this area of research is relatively new, there are no generally accepted protocols or quality data to be evaluated. Genomics, proteomics, and metabolomics appear to have potential for assessing organ specific toxicity, but relevant data for consideration have not yet been generated.

This Group suggested that the first step in the process is to gather all available data about the test substance and to accomplish as much as possible based on structure activity relationships. The second step is to conduct a basic cytotoxicity assay to assess *in vitro* toxicity. The third step is to evaluate the importance of metabolism (human cell lines should be used). Hepatic metabolism and energy metabolism are important issues as are the barrier functions—blood-brain, kidney, and liver. The Group's recommendation is to focus first on the issue of metabolism, by developing appropriate liver culture systems. When developing *in vitro* organ cultures, the greatest concern is whether or not the cultured cells continue to function appropriately.

Chemical Data Sets Breakout Group

Because Dr. Angela Auletta could not attend the ACATM meeting, Dr. Errol Zeiger provided an overview of Chemical Data Sets Breakout Group discussions and recommendations. The Group's task was to develop a chemical data set to be used in the validation of the *in vitro* test methods for acute toxicity recommended by the other Breakout Groups. The Group agreed that it was not the proper body and this was not the proper forum to select the chemicals for inclusion in the database. The primary task of the group would be to define the relevant characteristics of an appropriate chemical data set, and identify resources, approaches, and existing sources to be used in the development of the database.

Among the conclusions of the Group are that the classes of chemicals to be used for validation need to be defined by the test developer before the validation process starts, and that the chemicals selected need to be related to the purpose of the test and to the biological endpoint being evaluated.

The Group recommended that because the purpose of acute *in vitro* tests would be to measure, predict or estimate acute rodent (and eventually, human) toxicity, the reference LD50 data used should be of high quality. Other information concerning the reference LD50 data should include a measure of the variability of the value. For this reason, reference LD50 data should be taken, wherever possible, from tests and data that had been peer reviewed or where the tests were performed using accepted protocols, such as the OECD or EPA protocols, or where the tests were performed under GLP's. The Group recommended that ICCVAM undertake a study of existing databases and, where possible, determine the variation in the rodent LD50 values introduced by differences in protocols used by various regulatory agencies, and the between-laboratory reproducibility of the rodent LD50 test. Another recommendation is that ICCVAM convene an expert committee to identify a reference set of test chemicals from existing databases according to the following criteria:

- focus should be on commercially available liquids and solids with quality, reproducible LD50 data; any chemical that is highly reactive, a controlled substance, or highly corrosive should be excluded; and in addition to name and CASRN, the chemical database should include, for example, some alphanumeric structural designation like SMILES, or some other system, for sub-structure searching, LD50 data, other relevant acute toxicity information, physical-chemical parameters; the octanol-water partition coefficient, solubility, molecular weight, and pKa. Ultimately, the data selected for inclusion in the database should satisfy ICCVAM and ICCVAM guidelines. From that primary database of reference chemicals, subsets of chemicals would be selected for use in various validation studies, with selection to depend on the nature and purpose of the test. The Group recommended that good sources for the primary database include various databases at the U.S. EPA, FDA, and the NTP.

Priorities from the Workshop

Dr. Stokes discussed the priorities from the *In Vitro* Workshop. Publication of the workshop written report summarizing the conclusions and recommendations of the workshop is the first priority. The second priority is development of a guidance document about the 3T3 neutral red

uptake (NRU) assay, which will be developed by Drs. Fentem, Curren, and Liebsch. This guidance document will include a retrospective assessment of the existing 3T3 NRU toxicity data to ensure the data are predictive of the *in vivo* LD50. The third priority is to develop pre-validation and validation study strategies, which will begin as soon as the workshop report is completed.

In terms of the use of *in vitro* test method results for predicting human lethality, another recommendation at the meeting is that ICCVAM convene an expert panel to review the MEIC approach for estimating acute toxicity doses of chemicals in humans based on accidental exposure data, develop a broadly agreed upon standard approach for measuring acute toxicity parameters, and establish a program for retrieving existing human toxicity data.

Committee Discussion on Implementation of Workshop and Expert Panel Recommendations

The ACATM requested that a list of tests (endpoints), which are currently being used by various Federal agencies, be developed to assist investigators in establishing strategies for method development and requested also that the tests be grouped by area (neurological, reproductive, developmental, etc.). Dr. Stokes replied that ICCVAM would compile a list of the different testing requirements by endpoint and place it on the website. He listed several sources of test guideline information including the FDA Red Book 2000 as a source for FDA test methods used for foods and food additives, the U.S. EPA 870 OPPTS testing guidelines, and the Federal Hazardous Substance Act.

Dr. Stokes concluded by stating that the three most recent meetings organized by ICCVAM and NICEATM were very successful because of the involvement of several ACATM members familiar with the evaluation process and because of the leadership roles of many of the ACATM members at these meetings. He commended the participants in the three meetings for their strong commitment and many hours of preparation and participation.

Dr. Stitzel then asked if there were any public comments.

Public Comments

Ms. Sandler (PETA) stated that, unfortunately, it appeared that the *in vitro* tests discussed at the *In Vitro* Workshop (although more sophisticated than animal testing) must meet criteria not required by the LD50 tests and that none of the data presented at the workshop were new. She noted that European scientists are more advanced in developing alternative assays for *in vivo* acute toxicity compared to U.S. scientists and requested that the international scientific community be included in developing *in vitro* assays in the United States. Ms. Sandler stated that PETA perceives the 2- to 3-month time-frame for completion of the initial efforts by the Screening Methods Breakout Group as very reasonable and urged that the U.S. EPA immediately include guidelines in the High Production Volume Program on how to determine the starting dose for *in vivo* acute toxicity studies based on *in vitro* data. She recommended also that ICCVAM push the U.S. EPA for funds to be used for chemical selection and for a working group to be established to develop validation studies. Ms. Sandler stated that she attended the Non-Governmental Organization (NGO) meeting convened by the U.S. EPA to solicit comments

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from NGOs (meeting was held prior to the November OECD Joint Meeting) and learned that there is a proposed sub-lethal acute toxicity test, but that no one at the meeting seemed to know anything about it.

Ms. Amundson (Doris Day Foundation) expressed concern that no centralized place exists as a repository of toxicological information and explained that the ICCVAM Authorization Act of 2000 makes ICCVAM a repository for toxicological information. She commented also that resources should not be a problem because the U.S. EPA received millions of dollars over and above the Presidential budget for its endocrine disruptor program and that some of this money should be made available to ICCVAM to conduct its evaluations of alternative test methods.

Ms. Amundson completed her comments by inquiring about the individuals retiring from the ACATM and asked about the status of the nomination process to replace these individuals.

Dr. Stokes replied that the nominations for ACATM members, whose terms ended on September 30, 2000, have already been submitted to the Secretary, Department of Health and Human Services (DHHS); he stated that the NTP welcomes suggestions from anyone for advisory committee members that should be considered for future vacancies.

Dr. Rowan commented on the differences between the American and European perspectives on validating alternative toxicological methods. He stated that the American labs have produced a lot of new technology, but that the political process is much further ahead in Europe. He added that there is a need to get significant resources placed into an alternatives program within the Federal political system.

Dr. Stitzel adjourned the meeting at 4:50 p.m., acknowledging the exemplary membership of the ACATM and noting the dedication of the retiring members (Drs. Faustman and McClellan). She recognized also the outstanding work of ICCVAM and the NICEATM staff.