Scientific Advisory Committee on Alternative Toxicological Methods
National Institute of Environmental Health Sciences, Research Triangle Park, NC
August 12-13, 2003

Summary Minutes

The Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) met on August 12 – 13, 2003 at the National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina

SACATM Members Present

Daniel Acosta, Jr., Ph.D.
Rodger D. Curren, Ph.D.
Jack Dean, Ph.D. (chair)
Sidney Green, Jr., Ph.D.
Alan Goldberg, Ph.D.
A. Wallace Hayes, Ph.D
Stephen H. Safe, Ph.D.
Jacqueline H. Smith, Ph.D.
Carlos Sonnenshein, Ph.D
Martin L. Stephens, Ph.D.
Katherine A. Stitizel, D.V.M. (Day 1)
Peter Theran, V.M.D.
Calvin C. Willhite, Ph.D.

SACATM Members Absent

Nancy A. Monterio-Riviere, Ph.D
Katherine A. Stitizel, D.V.M. (Day 2)
Nancy Flournoy, Ph.D.

ICCVAM Ex Officio Members Present

George Cushmac, Ph.D. (DOT)
Patty Decot (DOD)
Kailash Gupta, Ph.D. (CPFC)
Jody Kulpa-Eddy, Ph.D (USDA)
Joseph Merenda (EPA)
Paul Nicolaysen, Ph.D. (NIOSH)
Alan Poland, Ph.D. (NCI)
Margaret Snyder, Ph.D. (NIH)
Leonard Schechtman, Ph.D. (FDA)
William Stokes, Ph.D. (NIEHS)

NIEHS Staff Present

John Bucher, Ph.D.
Kenneth Olden, Ph.D.
Christopher Portier, Ph.D.
William Schrader, Ph.D.
Barbara Shane, Ph.D.
Mary Wolfe, Ph.D.
Judy Strickland
Linda Litchard
Sally Fields
Debbie McCarley
Jerry Heindel, Ph.D.
Rajendra Chhabra, Ph.D.
Nupa Choksi
Brad Blackard
Michael Paris
Tracey Glazener

Other Federal Agency Staff Present

Joyce Royland, (EPA)
Daniel Casciano, Ph.D. (NCTR/FDA)
Suzanne McMaster (EPA)
Prasada Kodavanti (EPA)
Po-Yung Lu (DOE)

Members of the Public Present

Sara Amundson
Thomas Hartung Ph.D
Sue Leary
Raji Icucher Lapati
Jim Sherman
Amy Williams
Pat Crocket
Andrew Ballard
Kay McGovern
Mark Perry
Angela Licata
Daniel Marsman
Kristie Stoick
Brian Dell
Kathi Hoyer
Lynda Lanning
Federico Goodsaid
Errol Zeiger, Ph.D
Christina Inhof

August 12, 2003

I. Call to order and introductions

Dr. Jack Dean, chair, called the meeting to order at 8:35 a.m. on August 12, 2003, and asked the individuals seated at the table in the room to introduce themselves and give their affiliation. This meeting was taped for preparation of a transcript that would be used for summary minutes. The meeting was videocast from the NIEHS web site.

II. Welcome

Dr. Kenneth Olden, NIEHS Director, welcomed the SACATM and thanked them for attending the meeting. He told them that he would step down as director of NIEHS and NTP within the next 6-12 months or as soon as a replacement is found. He acknowledged the past and current leadership of the Environmental Toxicology Program and said he is supportive of the decisions being made. As director, his objective for the past 12 years was to bring the best science to bear on the decisions being made about public health. He felt confident that recruitment for his position would go smoothly. He added that NTP and NIEHS are separate and while historically the same person has served as director of both, they could be led separately. He noted his support for the scientific integration and cross-fertilization between NIEHS and NTP during this tenure.

Dr. Olden said the issue of alternative test systems/toxicological test methods is one of seven top NIEHS priorities and a good investment. He noted that many of these new test systems, such as genomics and proteomics, potentially will enable scientists to understand metabolic pathways and their interactions better and could lead to development of intervention strategies. He thanked the SACATM members for participating on the committee and providing advice.

Dr. Leonard Schechtman, NCTR/FDA, Chair of the Interagency Coordinating Committee on Alternative Methods (ICCVAM) welcomed the SACATM. On behalf of the ICCVAM he thanked them for their willingness to give their time and expertise to the committee. He said the ICCVAM looks to the SACATM for advice and direction for improving its processes, priorities, productivity, resources, efficiency, and efforts. ICCVAM along with the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) under the direction of Dr. William Stokes, provides a uniform mechanism for federal agencies to evaluate and consider

new, modified, alternative and scientifically sound methods for use in regulatory testing that may reduce, refine or replace animal use. ICCVAM has guidelines for the submission of candidate test methods and a formal process for evaluation of their validation status. This process facilitates the implementation by regulatory agencies of validated test methods that are responsive to animal welfare concerns. He noted that the national and international impact of ICCVAM has grown as a result of its strong, collaborative ties with the European Centre for the Validation of Alternative Methods (ECVAM) under the leadership of Dr. Thomas Hartung.

Dr. Christopher Portier, Associate Director of NTP, welcomed the SACATM, federal partners, and Dr. Hartung. He said his presentation on the activities of NTP and NIEHS related to alternative methods would be covered later in the agenda.

Dr. Wolfe read the conflict of interest statement for the SACATM.

III. Update on ICCVAM and NICEATM

Dr. William Stokes, NICEATM Director, welcomed everyone and thanked SACATM for its advice. He acknowledged attendance of the ICCVAM agency representatives and proceeded to provide an overview of ICCVAM and NICEATM activities since the December 2002 SACATM meeting.

Transmittal of ICCVAM Test Method Recommendations

Dr. Stokes said the ICCVAM Authorization Act of 2000 requires the Secretary, Health and Human Services, to transmit ICCVAM test recommendations to the appropriate agencies and the agencies have 180 days to respond; both the recommendations and responses are made public. The first test recommendations were for acute systemic toxicity. He said the Director of NIEHS transmitted them to the federal agencies in March on behalf of the Secretary and the agencies' responses are due in September. He added that responses are being posted on the NICEATM/ICCVAM web site as they are received. Dr. Stokes summarized the test method recommendations provided in the transmittal.

- The revised Up-and-Down Procedure for Acute Toxicity – the ICCVAM recommended this method as a valid replacement for the conventional LD50 test for hazard classification and concluded that its use would significantly reduce the number of f animals required for this testing requirement. The Organization for Economic Cooperation and Development accepted the method as a test guideline in December 2001 and EPA formally adopted it in December 2002.
    - The conventional LD50 method required 45 or more animals, while the revised Up-and-Down Procedure uses only 6-9 animals. However, it is a sequential test that can take up to 30 days depending on the relative toxicity of the chemical, compared to 14 days for the conventional LD50 test..
- *In Vitro* Methods for Assessing Acute Systemic Toxicity – ICCVAM and NICEATM organized an expert workshop that developed recommendations for research, development and validation studies for *in vitro* methods for estimating starting doses, assessing the toxicokinetics of a chemical, and predicting target-organ toxicity. Recommendations for selecting chemicals to use in validation studies for these types of methods were also provided.
    - ICCVAM recommended that cytotoxicity test data should be considered as one way to select an appropriate starting dose for acute oral toxicity tests. Use of these *in*

vitro tests in combination with the revised Up-and-Down Procedure may further reduce animal use by up to 40%.

- o ICCVAM concurred with the workshop recommendation to conduct validation studies on two standard cytotoxicity assays, one using a human cell system and one using a rodent cell system. NICEATM and ECVAM initiated this validation study in August 2002 with support from NIEHS and EPA, and in collaboration with ECVAM.
- o ICCVAM recommended that long-term research and development efforts should focus on improving *in vitro* systems for biokinetics, metabolism, and organ-specific toxicity that would facilitate accurate prediction of LD50s, symptoms of toxicity, and pathophysiological events.

· Guidance Document: Using *In Vitro* Data to Estimate *In Vivo* Starting Doses for Acute Toxicity – This document provides standardized protocols for two basal cytotoxicity methods: a rodent cell line (3T3 cells) and a human cell line (NHK). This guidance is based studies by ZEBET, the German Center for Alternatives in Berlin, who reviewed *in vitro* and *in vivo* data for 347 chemicals. These protocols have now been optimized for the validation study, and the updated protocols are available on the NICEATM-ICCVAM website.

ICCVAM/NICEATM Test Method Evaluation Activities

Dr. Stokes next briefly reviewed NICEATM activities related to evaluation of several test methods – *in vitro* estrogen and androgen receptor binding and transcriptional activation assays and *in vitro* dermal corrosivity assays. The *in vitro* endocrine assays are proposed for Tier 1 in the EPA endocrine disruptor screening battery. An independent scientific panel conducted an evaluation of the endocrine-related assays in May 2002 and concluded that there are no adequately validated *in vitro* methods for these types of test, and made recommendations for their standardization and validation. Dr. Stokes summarized ICCVAM's recommendations for the methods:

· Development of minimum procedural standards for these assays as a means for standardizing dose selection, dose spacing and concentration, and selection of controls. Dr. Stokes said Dr. George Daston, co-chair of the expert panel, would provide details about the panel's specific recommendations later in the agenda.

· Identification of recommended chemicals to include in validation studies for these assays. Dr. Stokes said this list was published in the Federal Register in October 2002 and public comment were considered in developing the final list.

Dr. Stokes noted that a goal is to establish minimum performance standards for these types of assays that can be used for judging the acceptability of future test methods that are based upon similar concepts.

Dr. Stokes next provided an update on Corrositex®, an *in vitro* corrosivity test, that was evaluated and recommended by ICCVAM in 1999. He said the ICCVAM working group and NICEATM have been involved in developing a generic test guideline for this method for submission to OECD, since OECD does not permit test guidelines for proprietary methods. NICEATM also released the draft ICCVAM performance standards for public comment and ICCVAM plans to finalize its recommendations for forwarding to the appropriate agencies later in 2003.

Dr. Stokes highlighted three other skin corrosivity methods previously validated by ECVAM – Epiderm™, Episkin™, and the Rat Skin TER. At EPA's request, ICCVAM and NICEATM developed minimum performance standards for generic versions of these assays and are

currently soliciting public comment on them. Recommended minimum performance standards will be forwarded to federal agencies later this year.

He said ICCVAM and NICEATM have also been evaluating results from *in vivo* dermal irritation and corrosivity tests. The goal of this evaluation is to develop performance criteria that can be used to evaluate *in vitro* methods proposed as replacements. Unfortunately, there is a paucity of high quality *in vivo* data, especially for dermal irritation, because it either has not been submitted to the regulatory agencies or it is proprietary. Dr. Stokes said NICEATM has asked industry and testing organizations to voluntarily provide this data, especially for chemicals with multiple test results and for chemicals with both human and animal data. A Federal Register notice was published that requested animal and human ocular and dermal irritation and corrosivity data.

ICCVAM Test Method Nominations and Submissions
Dr. Stokes briefly discussed ICCVAM's updated submission guidelines for test methods. The revised document describes the process for submission and nomination of test methods, the prioritization criteria used by ICCVAM for evaluating submissions and nominations and information about performance standards. He said ICCVAM would review public comments and make any final revisions to the document; its anticipated release is fall 2003.

Dr. Stokes updated SACATM on ICCVAM nominations and submissions. He said NICEATM anticipates two nominations – transgenic mice for carcinogenicity assays from the NIEHS and *in vitro* screening methods for ocular irritation. There are no submissions pending; however, NICEATM anticipates two submissions – *in vitro* androgen receptor and estrogen receptor binding and transcriptional assays. NICEATM also expects submission of an *in vitro* assay for ocular irritation for use in assessing the irritation of surfactants.

ECVAM Collaborations
Dr. Stokes identified three joint activities with ECVAM. The first is development of a justification for international guidance on the application of good laboratory practices (GLPs) to *in vitro* toxicity testing. There was a joint presentation of this justification to OECD in March 2003 that included the U.S. delegation of Dr. Schechtman, Dr. Amy Respin from EPA, and Dr. Stokes and the ECVAM delegation of Dr. Hartung and Dr. Sandra Coecke. He believes that OECD while initially reluctant to support development of this guidance, now appears supportive; the final decision will come in September.

The second activity is a collaboration of ICCVAM and NICEATM with ECVAM on an independent validation study for several *in vitro* dermal irritation methods. Representatives from ICCVAM and NICEATM have been invited to serve as observers on the ECVAM management team for this study. Dr. Stokes said NICEATM also conducted a retrospective review of available *in vivo* data to estimate the likelihood of the test underestimating dermal irritation potential.

The third collaboration is a validation study on *in vitro* methods for acute toxicity that started in July 2002. The study involves two U.S. laboratories and one in Europe. Phase I, which involved 3 chemicals, is completed and Phase II, which involves 9 chemicals, will end in September. Following this, the two protocols will be finalized and Phase III, which involves testing of 60 coded chemicals, will begin.

ICCVAM, NICEATM and ECVAM are planning three joint workshops – one on acute systemic toxicity, one on the validation of toxicogenomic-based methods, and one on good cell culture practices.

ICCVAM's Strategic Planning Initiative
Dr. Stokes said ICCVAM would begin a strategic planning process. The first objective will be to focus on identifying testing endpoints that should receive priority for future ICCVAM activities. The second objective will be to identify activities that would facilitate development, validation, and regulatory acceptance of alternative test methods, prioritize the activities, and focus on helping those identified as priorities move forward. He said the preliminary list of the top 5 testing priorities identified by the ICCVAM include: 1) acute eye irritation and corrosion, 2) acute dermal toxicity, 3) acute systemic toxicity, 4) chronic toxicity and carcinogenicity, and 5) reproductive and development toxicity.

Dr. Stokes identified other activities of ICCVAM and/or NICEATM. Both groups have liaison participants on the ILSI Biomarkers Subcommittee established in July 2003 to examine mechanism-based biomarkers of toxicity or safety. NICEATM continues to work with test method developers regarding nominations and submissions. Recent interactions are with two different developers on endocrine disruptor test methods.

Finally, Dr. Stokes identified scientific meetings where NICEATM and ICCVAM have made presentations: the Annual Meeting of the Society of Toxicology in March 2003 and the Annual Meeting of the Society for *In Vitro* Biology in June 2003. He acknowledged the ICCVAM Endocrine Disruptors Working Group, co-chaired by Dr. Marilyn Wind of CPSC and Dr. David Hattan from the FDA, the participation and contributions of agency representatives who serve on ICCVAM, the NICEATM staff including Debbie McCarley and Loretta Frye, and staff from the support contractor for NICEATM, Integrated Life Sciences.

Dr. Dean acknowledged the U.S. Surgeon General's recent appointment of Dr. Stokes as Chief Veterinary Officer for the Public Health Service Commissioned Corps.

Discussion
Dr. Stitzel raised several issues. First, training should be a high priority for moving alternative methods forward because often the regulatory agencies are not trained in how to deal with data from a new method. Second, it's important that methods be able to discriminate between positive and negative outcomes to ensure that the method is not indiscriminate. Third, she asked for clarification whether the validation study for acute oral toxicity would be a screening method. In reply, Dr. Stokes said the validation study for the two basal cytotoxicity methods is to determine their utility in estimating starting doses and that they are not proposed as replacement methods. However, a basal cytotoxicity assay is likely to be an essential component of any proposed *in vitro* replacement test battery. He also agreed that training on recommended alternative methods is important and noted that ICCVAM and NICEATM have sponsored training workshops for the LLNA and alternative *in vivo* and in vitro methods for acute toxicity. NICEATM plans to hold an implementation workshop for each new method recommended by ICCVAM.

Dr. Stephens asked that ICCVAM in developing its final list of top priority areas for alternatives consider not only current needs, but also anticipated testing needs at the national and

international levels. He supported continued collaboration with ECVAM. Dr. Stokes agreed and thanked him for this advice.

Dr. Curren addressed the activities on dermal irritation discussed by Dr. Stokes and asked him to comment on the strategy that NICEATM would follow to compare data for a chemical from an *in vitro* method against its performance using an *in vivo* method. Dr. Stokes replied that the strategy would depend upon what data are received and he envisions bringing any draft conclusions to SACATM for comment. He suggested that a workshop might be useful to develop scientific consensus on the issue.

Dr. Green asked Dr. Stokes to clarify the difference between generic performance standards and specific performance standards. Dr. Stokes said he would respond briefly since this topic would be covered tomorrow. Basically, the performance of the validated and accepted test method is used as a benchmark for accepting other test methods that are based upon the same structural and functional principles. The generic performance standards would include a subset of the chemicals used to validate the reference test method. The minimum performance standards would cover the essential structural, functional, procedural and performance elements of the protocol, and the comparable level of accuracy and reliability that should be achieved when the reference chemicals are evaluated.

IV. Update on Activities of ECVAM

Dr. Thomas Hartung, Head of ECVAM, provided an update of ECVAM activities and collaborations with NICEATM and ICCVAM since the December 2002 SACATM meeting. He provided data on animal use, which is 10 million animals per year, for toxicological or other safety evaluations in Europe. This use is comprised as 1% for cosmetics, about 10% for chemicals and 20% for quality control of biologicals, primarily vaccines and blood products. He noted some emerging legislative policies whose goal is to eliminate the use of animals to assess the safety of cosmetics and chemicals. The first is the European Union (EU) policy termed REACH, which requires testing of all existing chemicals produced at more than 1 tons per year (30,000 substances). The second is the 7th Amendment to the Cosmetics Directive that immediately bans animal testing of finished cosmetic products and in 10 years bans the marketing of cosmetics tested on animals for repeated dose toxicity, reproductive toxicity and toxicokinetics.

Dr. Hartung compared the expectations for reductions in animal use as a result of the legislation with a chart showing the distribution of animal use in toxicological evaluations per year. He said approximately 35% of current animal use is for assessments of acute systemic toxicity and fortunately many alternative methods have been successfully introduced. Dr. Hartung said ECVAM has restructured itself to address these legislative mandates and is forming steering groups that include outside experts to develop different strategies for alternatives. ECVAM estimates that the overall program for optimizing and validating these assays, excluding development costs, will cost about 150 million over 10 years.

Current activities include development of alternative methods, testing strategies and validation strategies and conduct of prevalidation/validation studies. ECVAM is creating a foundation of industry members to work with the task forces and project teams and hopefully provide substantial financing for the activities. Dr. Hartung described a program called ReProtect with 35 partners to develop a battery of alternative methods and testing strategies for reproductive

toxicity. He discussed the collaborative validation study for skin irritation being conducted with ICCVAM and NICEATM. He said three assays, Epiderm™, Episkin™, and SIFT are being compared; Phase I will assess current protocols with a limited set of 20 chemicals. Completion of all phases is projected for February 2005.

ECVAM is involved in analyzing the ECETOC database of *in vivo* data on 129 chemicals tested for skin irritation. The European Classification System analysis shows good intra-laboratory reproducibility and confirms the ICCVAM analysis. He said that most of the assays are more precise in predicting negative effects than predicting positive effects. He added that data from globally harmonized system contributes minimally since few chemicals are classified as mild irritants. ECVAM has appointed a task force to review the data available on *in vitro* and *ex vivo* tests for eye irritation. A validation study on pyrogen tests – six tests, which model the human fever reaction, was conducted through a consortium. ECVAM will submit the data sets to both ICCVAM and the ECVAM Scientific Advisory Committee (ESAC).

Dr. Hartung pointed out the ECVAM web site – http://ecvam.jrc.it - and invited everyone to register on-line to receive a monthly newsletter.

Discussion

Dr. Willhite asked Dr. Hartung about the feasibility of having an *in vitro* replacement for acute toxicity testing by 2007. He said this is the highest priority and ECVAM will hold a workshop in September to develop strategies and assess the feasibility of different approaches. Dr. Goldberg commented that the estimate of 10 million animals used annually is below what he expected since it's estimated that Europe represents 40% of animal use worldwide. Dr. Hartung said the data were for 1999 and added that it is the first time that the 50 member states provided individual data for the survey. In response to a question from Dr. Sonneschein, Dr. Hartung said he initiated a task force on endocrine disruption and one of its first tasks is to inventory current methods. Dr. Stephens complemented Dr. Hartung on ECVAM's proactive approach, including his activities to initiate task forces, stakeholder groups, and business and budget plans. Dr. Hartung said there is a large gap between the development of methods and their implementation for regulatory safety testing. Given the current legislation, ECVAM has no choice but to take an active role as the leader, coordinator and manager of these activities.

V. Federal Agencies Efforts in Alternatives

A. Test Method Development and Validation Activities at the U.S. Environmental Protection Agency

Dr. Joe Merenda, EPA, presented the overview and noted that EPA values the opportunity to participate in the ICCVAM. His talk covered information about how EPA uses toxicity test methods, the core principles that EPA considers in adopting new methods, collaboration efforts on test methods, highlights about EPA's test method use and development activities and finally future challenges. He said EPA is a science-based regulatory agency. The Office of Prevention, Pesticides, and Toxic Substances uses regulatory testing data from industry on a range of endpoints: human health, ecological effects, environmental fate, and efficacy of products. Other groups within EPA, such as the Office of Air and Radiation and the Office of Water, require limited toxicity testing. The Office of Research and Development examines or developments new methods; currently they are evaluating computational toxicology, including structural activity analysis and quantitative structure activity.

Dr. Merenda said the EPA follows a few *core principles* when considering whether to adopt a new test method: 1) the test method must be validated and based upon sound science, 2) its development and adoption must occur by an open and transparent process, 3) the method must address animal welfare issues, 4) it must promote international harmonization of test guidelines and 5) be practical for regulatory use. Three factors guide adoption of a test method; the EPA assesses its validation status, determines the applicability of the method for addressing key scientific questions, and evaluates its acceptability for regulatory use.

EPA has several collaborative efforts through ICCVAM and OECD. Dr. Merenda said the EPA is involved in the harmonization of international test guidelines and several validation efforts, including test methods for identifying endocrine disrupting agents. The EPA is actively involved in the ICCVAM process and participates on its various committees and working groups and co-sponsors workshops, *for example,* the training workshop for the Local Lymph Node Assay.

Next, Dr. Merenda provided some highlights of the EPA's test method development activities. First, he addressed the industrial chemicals area and the OECD screening information data set (SIDS) for high production volume (HPV) chemicals. As example, he cited the EPA's challenge to U.S. industry asking them to help fill gaps for six basic data areas covered by the SIDS. Subsequently, the EPA developed a voluntary children's chemical evaluation program for a subset of chemicals determined of high priority for exposure to children and pregnant women. EPA has worked to encourage public submission of existing data on these chemicals. To date, over 200 data summaries and test plans have been submitted to the EPA. In areas where data are not available, the EPA is using structure activity relationships, quantitative structure activity relationships, PBPK methods, and mechanistic data for preliminary assessments of the chemicals.

Dr. Merenda next said the EPA has licensing authority and responsibility for pesticides. The EPA must register new pesticide products, including their active ingredients and formulated products, before they can be marketed and sold in the United States. He said the pesticide active ingredients and formulations must undergo a battery of acute toxicity tests. These acute toxicity data impact EPA's regulatory decisions, *e.g.,* they determine whether child-resistant packaging must be used, labeling requirements, or whether the product will have restricted use. Therefore, EPA considers alternative test methods for acute toxicity a priority and is interested in ways to move forward with using alternatives. The EPA's strategy for alternatives is 1) to focus on some key areas: acute oral, eye irritation, and skin sensitization; 2) collaborate with ICCVAM to develop the data on the methods, *e.g.,* minimum performance standards, needed to ensure EPA's regulatory acceptance of them; 3) incorporate accepted methods into EPA's guidelines that sometimes requires development of technical aids or holding training workshops on the methods.

Dr. Merenda said the Office of Pollution Prevention, Pesticides and Toxic Substances is working with EPA's HPV/SIDS program to prevent redundancy of testing requirements or efforts in methods' development. EPA is interested in alternatives for its endocrine disruptor screening program. He said the EPA is continually challenged with determining how to incorporate non-animal methods more efficiently into its testing program. He briefly identified areas of EPA research that impact alternatives: computational toxicology and endocrine disruptors screening program – several laboratories are working on development and validation of methods in

collaboration with the regulatory program and. He said the National Academy of Sciences would advise the EPA on future directions for toxicology testing EPA.

Finally he identified some future challenges for the EPA in test method development:
- Alternative methods and non-animal methods for the endocrine disruptors screening program
- Identification of alternatives for ocular irritation testing
- Assembling reference data

Discussion

Dr. Curren asked whether EPA might be able to make public data that's been provided to it by industry. In reply, Dr. Merenda clarified that EPA does not own the data, but receives data for regulatory review and there are a number of restrictions governing the data's use. He said that EPA has on occasion negotiated with registrants for public accessibility to data and this should continue to be pursued. Second, he said EPA might be able to use the data internally and make comparisons, *for example,* between test systems and release that information in a way that would not reveal proprietary information; EPA legal counsel need to evaluate this issue. Finally, he said EPA has some data that are only available in hard copy.

Dr. Hayes asked if the use of alternatives had reduced the number of animals needed in the HPV and voluntary child chemical alternative programs. Dr. Merenda said he did not think so, but would try to find out.

Dr. Goldberg mentioned participating in an ILSI/HESI meeting about pesticides. He said industry uses a battery of *in vitro* and short-term tests as screens to determine what pesticide products to move forward for regulatory testing. The battery appears to predict product safety. He felt that industry and EPA should evaluate the battery as a possible replacement for the required regulatory testing. Dr. Merenda said he was not aware of that information and said he would follow-up with EPA staff.

Dr. Stephens proposed a strategy for EPA to address animal use: EPA would determine animal use across the agency for different endpoints, identify changes in the animal use profiles over time, and use that information to determine priority areas for alternatives. Dr. Merenda said this would be a challenge for the agency given the number of different programs, but he would take the suggestion to EPA staff. He added that the pesticide program has undertaken a less analytical approach by looking at specifically at acute toxicity testing and endorses eye irritation as a priority. Dr. Dean said it would probably be easier for industry to compile the information about animal use than EPA. Dr. Merenda said he would raise this issue with the Pesticide Program Dialogue Committee.

B. A Systeomic Approach to Toxicogenomics

Dr. Dan Casiano, NCTR Director, presented an overview about NCTR's efforts to develop a systematic approach to toxicogenomics. He defined systeomics as an integration of genomics, proteomics, and metabonomics to solve a biological/toxicological problem. Toxicogenomics is a new scientific subdiscipline that combines the emerging technologies of genomics, proteomics and bioinformatics to identify and characterize mechanisms of action of known and suspected toxicants. NCTR is interested in the fidelity of DNA replication, the activities associated with a toxicant affecting genomic expression, the impact on the expressed gene to produce functional

protein, and the changes in metabolic profiles from expression of the protein. Dr. Casciano presented the NCTR's strategy - in the short-term to develop a gene expression database in surrogate organisms and in long-term to develop a validated gene expression database in humans. He said the NCTR has put in place the necessary infrastructure to carryout this effort and briefly described five centers. The Functional Genomics Center studies gene function using DNA microarray technology. Currently it has a core center to standardize molecular, analytical and informatic tools. The types of genes being studied include those involved in drug metabolism, cell cycle and apoptosis, stress response, and inter-and intra-cellular communication. Current activities include developing a rat mitochondrial chip to investigate the relationship between mitochondrial gene expression and mitochondrial protein expression, defining gene expression in aging Fisher 344 rats – the strain used in NTP studies, comparing circadian rhythm gene expression in F344 and CD rats, and using laser capture microdissection to probe organ-specific gene expression.

N ext, Dr. Casciano discussed work being done by the Structural Genomics Center. The group is interested in identifying single nucleotide polymorphisms and determining potential susceptibility to cancer of human populations by identifying genes that predispose people to cancer, detecting elevations of risk resulting from exposure to known toxicants, and identifying genes that modify cancer survival. He said several molecular epidemiology studies are underway.

Dr. Casciano said the Toxicoinformatics Center is providing software infrastructure and analysis capabilities for the studies underway by the other centers. The database housing the gene expression data is called ArrayTrack. NCTR has worked with ILSI, the European Bioinformatics Institute, and the National Center for Toxicogenomics at NIEHS in defining the database for toxicology information, MIAME/Tox. He demonstrated ArrayTrack and said it is publicly accessible at http://weblaunch.nctr.fda/jnlp/arraytrack. A goal is to develop the structure activity toxicant library, Toxicoinformatics Integrated System. He said NCTR has a current collaboration with the Center for Drugs and Merck to evaluate glitazone and has a project on acetaminophen that parallels studies underway by NTP.

In closing, Dr. Casciano said the alternative methods described will result in a reduction in animal use and provide information on the utility of surrogate organisms for understanding human genomic profiles. He acknowledged the persons involved in this effort.

Discussion

Dr. Green asked about the validation of these methods and their incorporation into the testing requirements by specific product centers at FDA. Dr. Casciano said the product centers are aware of this project and are collaborating on it. *For example*, NCTR is collaborating with 1) the Center for Devices and Radiological Health on the diagnostics tools, 2) the Center for Drugs on systems, platforms, statistical tools, informatic data mining tools; this center is using ArrayTrack, and 3) the Center for Food Safety and Applied Nutrition on microarrays for evaluating food safety. Dr. Safe complemented Dr. Casciano on the work being done and noted that this type of technology would likely be more informative in the long run than current *in vitro* methods such as the estrogen and androgen receptor binding or transcriptional activation assays.

Dr. Sonneschein questioned the rationale for developing the gene expression database in Fisher 344 and Sprague Dawley rats. Dr. Casciano replied that the Fisher 344 rat is the strain

used in NTP studies of FDA nominated chemicals. They want to have gene expression data to interpret disease outcomes resulting from exposure to FDA-regulated chemicals and hopefully relate this information to humans. The Sprague Dawley rat is the animal most frequently used by industry.

Dr. Stitzel congratulated Dr. Casciano on this project. She asked if the NCTR and NIEHS databases for genomics are compatible. Dr. Casciano said NCTR interacts regularly with National Center for Toxicogenomics at NIEHS.

C. Alternative Toxicological Test Methods

Dr. Jodie Kulpa-Eddy, USDA, spoke about the alternative toxicological methods used by the USDA and their efforts in alternatives. She briefly described the agency's organization and said the Under Secretary for Marketing and Regulatory Programs oversees the regulatory program, which resides primarily within the Veterinary Service. The Center for Veterinary Biologics is responsible for regulating veterinary biologics, including vaccines, bacterins, antisera, and diagnostic kits, to ensure that they are pure, safe, potent and effective before being released to the public. Dr. Kulpa-Eddy said purity testing, which detects viable bacteria and fungi in killed programs and extraneous viable bacteria and fungi in live products, does not require testing in animals, but safety testing does.

She said the mouse safety test is the most common test for determining the safety of veterinary products; it requires 8 mice in the 7-day test and if it's harmful to the mice, then a guinea pig safety test is done. Poultry products are tested in chickens. Dr. Kulpa-Eddy noted that the United States, Japan and the European Union are participating in the International Cooperation on the Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products to reduce the number of animals used in safety testing. The Target Animal Safety Testing Group is working on developing guidelines for minimum requirements for determining product safety. The goal is one test for safety that is accepted by all groups.

Dr. Kulpa-Eddy briefly discussed potency testing. The purpose of potency testing is to provide assurance that the active component(s) required for efficacy of the vaccine are present in a concentration and state that's been shown to be effective in the host animal. She said each serial released to the public must undergo testing by the manufacturer. Potency testing has the highest animal usage and therefore the most potential to reduce animal pain and distress by incorporating the 3Rs into the regulatory requirements.

Dr. Kulpa-Eddy provided a historical perspective of potency testing. In the 60s and 70s all vaccines required vaccination and challenge to the target species or surrogate laboratory animal species prior to the serial's release. Potency testing of modified live virus vaccines was replaced by quantification of the live organisms (titration). This reduced animal usage greatly and was the first example of *in vitro* potency testing used by the biologics program. Next the *master seed* principle was introduced whereby if a virus or modified live virus is shown to be effective in protecting the host animal, then after 3 years it is re-quantified and the master seed can be used to propagate more serials for release of vaccine to the public. Dr. Kulpa-Eddy noted that approximately two-thirds of the viral vaccines could be tested by titration methods. From 1980s to present the regulation changed from virus titration in lieu of animal testing to *in vitro* tests for both viral and bacterial viruses. In 1997, the regulation was revised to allow potency testing by including information on relative antigen content determined by ELISA

(enzyme linked immunosorbent assay). This *in vitro* method potentially could be a complete replacement for the animal vaccine challenge test; however, there are several drawbacks: it measures only one or a limited set of antigens and fails to evaluate other protective antigens or vaccine components, the level of active agent cannot be determined, adjuvant may interfere with the assay and unless monoclonal antibodies are used, it typically does not differentiate biologically active from denatured antigen.

Potential candidates for validated *in vitro* assays include: *Leptospira* bacterins - a hamster vaccine challenge test, *Clostridium chauvoei* bacterin - a guinea pig vaccinate-challenge test, and *Erysipelothrix rhusiopathaie* bacterin - a mouse protection relative potency test. These tests require vaccination and challenge on the serial and a standard, so twice the number of animals is used. Dr. Kulpa-Eddy said Code 9of Federal Regulations requires manufacturers to submit a test result to the USDA for every serial that they want to make public. She showed estimates of animal use based upon determinations of the number of mandatory tests conducted by manufacturers in 2001-2002. Animals used per year were: *E. rhusiopathaie* test: 120,000 mice, *Leptospira*: 40,000 hamsters, and *C. chauvoei*: 18,000 guinea pigs. If validated *in vitro* tests were available, animal usage would be dramatically reduced. She said some manufacturers have replaced the animal vaccinate-challenge tests with alternatives; however, because it's confidential business information, it is not public.

She mentioned several refinement methods – a rabies vaccine challenge tests conducted in mice and swine *Erysipelas* bacterin that uses the mouse challenge test. The Center for Veterinary Biologics encourages manufacturers to submit alternative methods for animal potency tests; however, because they are confidential business information, they are not public.

Discussion

Dr. Stitzel thanked the USDA for providing their presentation. Dr. Theran asked about the timeframe for having the alternative methods for *Leptospira* and *Erysipelothrix* available. Dr. Kulpa-Eddy said the methods exist, but there is insufficient funding available for their validation against the host animal. Validation of the *Leptospira* test will begin soon due to some available 2003 funds. Dr. Goldberg asked if industry is aware that a score of 2 in the rabies vaccine challenge test is sufficient to end testing and Dr. Kulpa-Eddy said USDA tries to communicate with industry whenever possible; two venues are a meeting held annually in Ames, Iowa and site inspections of the biological manufacturers' facilities.

D. National Institutes of Health and the Pursuit of Animal Welfare

Dr. Margaret Snyder discussed animal welfare at the National Institutes of Health (NIH). Her talk focused on three points: 1) the Public Health Service Policy and the U.S Government Principles promote animal welfare, 2) education and training support animal welfare and 3) new scientific and improved technologies promote alternatives. She said the NIH mission fosters an environment applicable not only to health and human welfare, but also to animal welfare. Dr. Snyder provided a chronology on animal welfare noting that as early as 1904, the NIH, then called the Hygienic Laboratory, had policies on animal welfare. In 1930, the Hygienic Laboratory became the NIH. In the mid-late 1940s, laboratory animal sciences emerged at the NIH with recruitment of a veterinarian and later a veterinary pathologist. She noted that before the 1950s animal husbandry was not well monitored, and in 1950, the NIH Director, Dr. Rolla Dyer, put into place rules regarding the use and care of animals in research. In 1943 two

discoveries – the process for growing cells in culture by Wilton Earl from NCI and the development of medium for growing multiple tissues in culture by Virginia Evans– helped set the stage for *in vitro* research. In 1950, the fathers of laboratory animal sciences known as the Chicago 5 convened a meeting to discuss animal welfare and use of animals in research. This group continued as the Animal Care Panel and later became the American Association for Laboratory Animal Science (AALAS). Dr. James Shannon, NIH Director from 1955-1968, helped increase the NIH budget 15-fold and changed its focus from science to health.

Dr. Snyder pointed out that the Institute for Laboratory Animal Research (ILAR) of the National Academy's of Sciences was founded in 1952 to provide authoritative reports on subjects of importance to those persons involved in animal care and use, to serve as a clearinghouse for information about animal resources and to develop and make available scientific and technical information on laboratory animals and other biological research resources. In 1959, the NIH Physiology Training Committee (now part of the National Institute of General Medical Sciences) initiated extramural funding for training in laboratory animal medicine. Dr. Snyder said the Animal Care Panel (noted earlier) prepared the first guide for laboratory animals, which was published in 1963 and was funded by NIH. In 1967, the idea of animal models of disease became more focused and the Institute for General Medical Sciences sponsored a workshop on comparative medicine with emphasis on selection of appropriate animal models.

Dr. Snyder outlined the history of NIH policy on animal welfare. Following publication of the Animal Welfare Act in 1966, the NIH in1967 issued a policy on animal care and use that put responsibility on the investigator to exercise precaution and ensure proper care and humane treatment of laboratory animals. In 1971 through its Institutional Relations Section of the Division of Research Grants the NIH refocused its policy to place responsibility on the institution to carry out the principles of animal welfare on behalf of the NIH. It required all institutions receiving NIH funding to submit the first assurance form and either be accredited by AALAC or establish a committee to evaluate the care of all warm-blooded animals used in research. Dr. Snyder said this NIH policy became a PHS policy in 1973. In 1977, ILAR published *The Future of Animals, Cells, Models, and Systems in Research, Development, Education and Training,* which was co-sponsored by the NIH and multiple animal welfare groups. That report looked at the state of science and the pursuit of animal welfare and alternatives. In 1979 the PHS policy was revised to require a committee to review the care of live vertebrates. In 1982, NIH developed specific instructions for reviewers of grants and contracts to consider the appropriateness of the propose species, the number of animals being used, justification for their use and the quality of proposed animal care.

In 1985, federal agencies accepted the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training. Those principles address the broad concept of alternatives. Additionally in 1985 Congress passed the Health Research Extension Act. In 1985 NIH policies were further revised. The institution's animal committee was now officially called an Institutional Animal Care and Use Committee (IACUC). Additionally, the institution's veterinarian was now required to have laboratory animal experience. The IACUC was to be appointed by the CEO. The Institutional Official (IO) was to make commitments on behalf of the institution that the requirements of the PHS Policy will be met, and the IACUC's composition was now defined to include a chair, veterinarian, a lay member, a non-affiliate member, and a practicing scientist.

In 1998, Congress pushed for a reduction in regulatory burden for extramural scientific research. This led to discussions and a concerted effort to "harmonize" where possible, the requirements for the Office of Laboratory Animal Welfare (OLAW), the Animal and Plant Health Inspection Service of the USDA, and AAALAC. Dr. Snyder said today animal welfare is considered "part of the environment" in conducting research. OLAW in addition to serving as the compliance oversight office for animal welfare for research supported or conducted by any component of the Public Health Service, also serves as a resource for educational materials and programs that assistance institutions in understanding compliance with the PHS policies.

She highlighted a new ILAR report, *Guidelines for the Use of Animals in Neurosciences and Behavioral Research*. Dr. Snyder pointed out that the NIH Roadmap, an initiative led by Dr. Zerhouni, NIH Director. The Roadmap has 3 broad themes that should meet future scientific challenges: New Pathways to Discovery, Research Teams of the Future, and Re-engineering the Clinical Research Enterprise. She believes the theme of *New Pathways to Discovery* is the richest in supporting development of alternative test methods.

In summary, Dr. Snyder pointed out that animal welfare policy has evolved over time taking small progressive steps toward a good set of policies and regulations. NIH has supported training and education that advance animal welfare and NIH supports the development of new science and innovative technologies, which will further promote the development and implementation of alternatives.

Discussion

There were no questions directed to Dr. Snyder.

E. NCI and Transgenics

Dr. Alan Poland, NCI, noted that the NCI has invested considerable time and resources in developing the Mouse Models of Human Cancer Consortium (MMHCC). He showed the web site and provided overview information about the MMHCC.

Dr. Poland introduced Dr. Raju Kucherlapati, co-chairman of the consortium. MMHCC is composed of 17 groups that include about 80 investigators from 40 U.S. institutions as well as investigators from the NCI intramural program. Dr. Kucherlapati said he is also a member of the NIEHS Comparative Mouse Genetic Centers Consortium (CMGCC). He said the goal of the MMHCC is to develop genetically modified mouse models that can serve as models for human cancer. The goal of the CMGCC is to understand the role of human genetic variants in health and disease. He identified the two approaches being taken by the consortia: to generate mice 1) where pathways known to be involved in tumor formation are disrupted or 2) containing mutated human genes known or implicated in predisposition for human cancer. Dr. Kucherlapati said the work of the consortia is providing valuable tools for understanding the biology of cancer and other disorders. He identified as few projects of the MMHCC.

- Dr. Doug Hanahan, University of California in San Francisco, has developed models for insulinomas, identified the properties of angiogenesis in these tumors, and is studying the genes involved in angiogenesis and the responsiveness of these tumors to drug therapy.

- Dr. Tyler Jacks, Massachusetts Institute of Technology, has developed a mouse model for lung cancer based on activation of the Kirsten-*ras* gene that shows characteristics of human lung cancer.
- Drs. Ron Depinho and Lynda Chin at Dana Farber Cancer Institute have developed mice to investigate the maintenance of tumors and describe the role of the Harvey-*ras* gene in this process. Importantly, tumor regression appears linked to inhibition of angiogenesis.
- Dr. Kucherlapati's group is studying the role of DNA mismatch repair genes in colon cancer. They have generated mouse models for hereditary nonpolyposis colon cancer and familial adenomatous polyposis and learned that mutations in MSH2, MLH1 and MSH6 can lead to a cancer predisposition phenotype in both mice and humans.

Discussion

Dr. Green said the rationale for how transgenic models fit within alternative methods is not clear and asked that it be explained. In reply, Dr. Poland said these models could serve as alternatives to the mouse strain used in the standard NTP bioassay and also provide insights about what might be the best model to use. In light of the complexity of mouse models developed by the NCI consortium, Dr. Poland felt that the use of two models in the NTP Bioassay (v-H-Ras and p53 KO) was simplistic and not adequately considered. Further, the correlation of results on compounds using the standard bioassay and these two transgenic strains was rather disappointing, r= 0.84. Dr. Snyder said it's anticipated that the actual number of animals per experiment would decrease by providing a better test model. Dr. Olden added that he feels these models will ultimately reduce animal use, but also be more informative by providing mechanistic information about pathways for disease etiology and/or mechanisms for toxicity. Dr. Stitzel agreed and thought these models could be more precise than standard rodent models and might reduce the number of studies needed to understand the toxicity of a chemical.

Dr. Goldberg asked Dr. Kucherlapati to identify the criteria that determine that transgenics are validated models for human disease and to provide guidance on how to address animal welfare issues associated with use of these models, such as multiple tumors, shortened life spans, and potential pain. Dr. Kucherlapati said the criteria currently being used are: first - whether the gene has a role in human cancer, second - whether the genetic modification observed in the mouse (*e.g.,* point mutation, null mutation, etc.) mirrors that found in the human population, third - whether tumorgenesis mimics that observed in humans, and fourth - whether the biochemical pathways affected in the mice are comparable to what's observed in humans. He agreed with Dr. Olden that these models would be more informative and potentially reduce the number of animals used and the length of study. Dr. Casciano added that these systems also allow for development of *in vitro* systems using cells isolated from the mice, which would reduce animal use.

Dr. Sonneschein asked Dr. Kucherlapati to address how well the transgenic models actually mimic human carcinomas. In reply, he said the early models were based upon inactivation of a gene in the germ cell line and generally are not good mimics for human cancers; however, more recent models are based upon somatic mutations in particular tissues and these tumors mimic those seen in humans.

Dr. Theran commented that the number of animals being used in this type of research has increased significantly. He believes that consideration of transgenic models as alternatives is

because of the ability of these models to provide potentially more scientific information per animal. He added that the laboratory animal medicine associated with transgenics is a challenge to laboratory veterinarians and becoming a separate specialty.

F. NIEHS/NTP Research Activities to Replace, Reduce and Refine the Use of Animals in Toxicology

Dr. Christopher Portier identified the two NIEHS research programs – extramural, which includes grants and support for NTP contracts, and intramural, which includes the Environmental Toxicology Program and NTP Operations. He said the Division of Extramural Research Training uses primarily grants and contracts to support extramural research and training in environmental health: many projects on alternatives are funded as Small Business Innovative Research grants (SBIRs). The Division of Intramural Research conducts high risk and/or long-term research on environmental health and serves as a national resource on issues related to environmental health. He outlined the purpose of the NTP: a multi-agency federal research program whose focus is to coordinate and collaborate on toxicological testing programs with the Department of Health and Human Services, develop data to strengthen the science base in toxicology, develop and validate improved testing methods, and communicate information about potentially toxic substances to a wide constituency. He pointed out three NIEHS programs: NICEATM, the National Center for Toxicogenomics (NCT) and the Environmental Genome Project. NIEHS is adding to NICEATM with additional staff and renewal of the support contract. NCT is using genomic technology to improve our understanding and detection of toxic substances. The NCT consists of an NIEHS component and a consortium of five academic research centers; the NTP is a partner in the NCT and the consortium. NCT research includes studies conducted at NIEHS, academic institutions and contracts. Dr. Portier noted that the NCT is heavily involved in methods standardization and validation and the NTP is working closely with NCT to ensure coordination of efforts in both genomics and toxicology. A long-term goal is to predict the toxicity of an agent by toxicity fingerprinting – using genomic tools to identify patterns of gene expression, protein activity, metabonomics, etc. Dr. Portier said the Environmental Genome Project is gathering in human populations baseline data on the variation and frequency of genes associated with environmentally related diseases. These genetic variants are being studied *in vitro* in ways analogous to those being done *in vivo* with transgenic mice in order to understand the role of individual genes in disease etiology.

Dr. Portier pointed out that NIEHS research on alternatives addresses the 3Rs.

- Reduce the number of animals used through evaluation of multiple toxicity endpoints in the same study, research on optimal experimental designs by statistics and mathematics, improved access to data generated in studies, and by using alternative models such as genetically modified animals.
- Refine the number of animals used through development of non-invasive imaging methods, methods applicable for studying humans or human tissues, and invertebrate models.
- Replace animals with other methods by mechanism-based research, QSAR research, validation studies, and computational modeling.

Dr. Portier identified areas of alternatives research at NIEHS.

- Small Business Innovative Grants (SBIRs) – 5 grants – *for example,* development of a rapid, high-through-put deletion assay for mammalian cells
- Development of non-mammalian models as replacement methods for evaluating toxicity – 6 grants - *for example*, grants on zebrafish and *C. elegans*

- Development of *in vitro* assays – 15 grants - *for example,* development of *in vitro* model for allergy screening and recombinant bioassays for endocrine disruptor screening
- Technology development – 5 grants - *for example,* development of an *in vitro* biochip device for toxicology testing
- *In vitro* research – 7 grants - *for example,* development of an enhanced local lymph node assay
- Transgenic research – 131 grants *for example,* marine, p53 models, *Xenopus* models
- Intramural research in 2002 - *for example,* research on development of functional toxicology screens and yeast-based assays for apoptosis and basic research on computational chemistry and molecular modeling and DNA replication fidelity across species
- Research and development contract support for NTP studies and international collaborations - *for example,* with the Ramazzini to make data from their toxicology studies available

In summary, Dr. Portier said the NIEHS and NTP efforts on alternatives include broad-based research efforts in basic science, improved testing methods, assay development and assay validation and a mechanism-based approach to toxicology that builds upon *in vitro* to *in vivo* linkages.

Discussion

Dr. Stephens thanked Dr. Portier for the breadth of his presentation and said SACATM would be interested in similar information from the other ICCVAM agencies. Dr. Curren asked whether the public could gain access to summary information about SBIR projects. Dr. Portier asked Dr. Jerry Heindel to respond and he said the CRISP database contains information on all NIH-funded grants and is publicly accessible through the Internet. Dr. Hayes asked how SACATM might help with promoting validation efforts. Dr. Portier said SACATM could be important in helping set priorities about which assays to move forward to validation and which areas need alternatives. Dr. Goldberg noted a lack of effort on translating methods from basic research into tools useful by regulatory agencies in testing and screening. Other SACTAM members agreed. Dr. Theran pointed out that one approach would be to identify those areas where a large number of animals are being used and the use is associated with animal pain and suffering.

Public Comment

Sara Amundson, Doris Day Animal League, said the meeting is one of the most informative she'd attended in the past eight years since the previous advisory committee was constituted. She directed two points toward the federal agencies. First, she said the other agencies should identify priority areas for alternatives as the USDA did. Second, she asked the other agencies to follow the example set by the NIEHS in reviewing its total portfolio on alternatives and providing comprehensive, agency-wide information about their efforts. She asked for information about funding for ICCVAM activities through NICEATM for fiscal years 2003 and 2004. She provided the SACATM with a copy of the report language from the Senate's Labor HHS and Education Subcommittee and noted that increased funding of ICCVAM activities to assess the validation of specific alternative methods is one of five priorities identified for NIEHS. She encouraged the SACATM to develop a proactive 5-year strategic plan for alternatives.

Discussion

Dr. Portier supported ICCVAM identifying priority areas for alternatives. Several members provided comments in support of SACATM giving input on priorities for resource allocations. Dr.

Dean suggested that SACATM form a subcommittee to identify gaps and areas for priority; however, he said the subcommittee would need information from the agencies on their priorities or mandates, animal use, and areas for animal pain and distress. Dr. Stitzel said development of alternatives should focus not just on endpoints, but also on comparing toxicity mechanisms for commonalities. Dr. Stephens said NIEHS has been a major supporter of efforts on alternatives and proposed that ICCVAM agencies contribute to a common pool for funding alternative projects. He also proposed that the agencies routinely collect information about their efforts on alternatives, similar to the NIEHS presentation, and identify their short-term and long-term needs. Dr. Willhite suggested that the Senate Subcommittee's language for NIEHS be expanded to other agencies to promote widespread support for alternatives.

Dr. Portier noted that NICEATM has not received any nominations for methods validation and asked SACATM for recommendations on how to get nominations. Dr. Hayes asked if the nomination process would allow the nomination of methods needing validation and Dr. Portier answered yes. Dr. Portier said NIEHS would seek advice from SACATM before moving forward with a validation. Dr. Stokes said funds are available for NICEATM to review the information provided on a method and its potential regulatory applicability, and if necessary supplement that information with details about additional validation studies that might be necessary. Next, the nomination would be taken to the ICCVAM for comment on what priority it should receive. Next, this information would come to the SACATM for comment. In reply to a question from Dr. Hayes, Dr. Portier said the NIEHS would be interested in learning what resources the USDA needs to complete validation of the *in vitro* vaccine method.

Dr. Green asked if ICCVAM might develop a template for collecting information about alternative using a common format that includes information on title, fundings, etc. Dr. Stokes said that NICEATM had sent a survey to the agencies on behalf of ICCVAM asking them to delineate test methods currently in development or undergoing validation. Once collated, the information would be posted on the NICEATM web site with contact information for the principle investigator. He said NICEATM is also surveying the agencies for areas of high priority.

Dr. Portier pointed out that the letter inviting the ICCVAM member agencies to present information about their efforts in the development and validation of alternative methods was flexible in what the agencies wanted to present. He suggested that SACATM might define *alternative assay* in order to provide context and consistency in the information that the agencies might provide. Dr. Curren suggested that industry be involved in discussions on needs and priorities for alternatives. He said it would be advantageous for industry to collaborate with others on developing and validating methods for safety testing. Dr. Hayes added, however, that the current system doesn't promote validation of methods used by the company internally because it limits their range of utility. Dr. Goldberg added that regulatory guidelines often require additional testing in animals because of the limits on use set for the *in vitro* alternative methods. Dr. Portier said he supports working with industry groups that have proprietary information that might be important to future validation efforts. He also looks forward to input from SACATM about directions for the future. He thanked the agencies for their presentations.

The first day of the meeting was adjourned at 4:57 p.m.

August 13, 2003

Dr. Dean, chair, welcomed everyone to the second day of the SACATM meeting. Persons seated at the table and observers in the room introduced themselves. Dr. Wolfe read the conflict-of-interest statement and noted that the meeting was being videocast over the NIEHS Internet site. Dr. Stitzel was absent for day 2 of the meeting.

VI. Application of Good Laboratory Practices to *In Vitro* Studies

A. Report on OECD Consultation Meeting on the Application of GLPs to *In Vitro* Studies

Dr. Schechtman, NCTR, reported on a meeting held in Paris in March 2003 with OECD on the application of Good Laboratory Practices (GLP) to *in vitro* studies. The goal was to discuss the need for guidance on the application of the principles of GLPs to *in vitro* studies. Representatives from ICCVAM and ECVAM attended the meeting and presented oral comments justifying the need for developing additional guidance to the OECD Working Group on GLP.

Dr. Schectman outlined the scope of the GLP principles. He noted that all non-clinical health and environmental safety studies used for registering or licensing products and materials, such as pharmaceutical, pesticides, food and feed additives, cosmetics, veterinary drugs, medical devise, industrial chemicals, must be conducted under GLP. ICCVAM and ECVAM felt that the increased use of *in vitro* methodologies, especially where those tests would be conducted for regulatory purposes, necessitated the need for GLPs. He added that ICCVAM and ECVAM believed this guidance should be a separate document and not a revision of the *OECD Principles of Good Laboratory Practice* or the *OECD Consensus Document on the Application of GLP Principles to Short-term Studies*. Such guidance would ensure the quality and reliability of *in vitro* methodologies and the interpretation of their data, help interpret how current GLP principles would be applied to *in vitro* studies, ensure confidence in data generated by studies using these types of methods, and be useful for study directors, auditors and regulators. Dr. Schechtrman said the document would be separate from guidance for *in vivo* methods and would not apply to guidance for validation studies of alternative, nonanimal methods. A future goal would be for the GLP Guidance to be incorporated into Best Practices Guidance, such as the *Good Cell Culture Practices* document, and into validation studies.

Dr. Schechtman briefly outlined the meeting. He noted that previous presentations to the OECD Working Group on this topic had been unsuccessful in gaining support for developing specific guidance for *in vitro* methodologies. Discussion on the first day covered the need for developing further guidance on the application of GLPs to *in vitro* methodologies. Dr. Stokes and he gave a presentation, *ICCVAM/ECVAM Justification for the Development of an "OECD Guidance Document on the Application of Good Laboratory Practices Principles to In Vitro Testing."* Drs. Coecke and Hartung presented the ICCVAM/ECVAM joint proposal, *The Application of the Principles of Good Laboratory Practices to In Vitro Toxicological Studies.* Dr. Schechtman said the goal of these presentations was to identify the benefits in having a specific guidance document for *in vitro* methodologies and to show how it would supplement and complement the existing OECD GLP guidance.

Dr. Schechtman noted that there was considerable discussion among the OECD Working Group members regarding whether this specific guidance for *in vitro* methodologies is needed. There was disagreement among the members whether the document is necessary. Some members felt no specific *in vitro* GLP guidance document is needed because those issues would be covered in *OECD Principles of Good Laboratory Practice* and/or *Consensus Document on the Application of GLP Principles to Short-Term Studies*. Others thought that the existing principles are not specific enough to be applicable to modern *in vitro* methodologies, such as genomics and proteomics, and that OECD should be more proactive on this issue. The outcome of this discussion was support for a separate document on GLPs for *in vitro* methodologes. The ICCVAM and ECVAM representatives offered assistance in helping draft the document.

The second day was a closed session of the OECD Working Group to further discuss the issue of guidance for *in vitro* methodologies and whether to endorse development of a guidance document. The OECD Working Group decided in favor of drafting an OECD consensus document for *in vitro* methodologies and invited ICCVAM and ECVAM input to participate. Dr. Schechtman said the document, *Draft Outline of an "OECD Consensus Document on the Application of GLP Principles to In Vitro Test Systems and In Vitro Studies*, would be submitted to the OECD Working Group at its meeting in September. It addresses issues such as definitions, responsibilities of the test facilities, and standard operating procedures of the test systems, quality assurance, and record keeping. He hoped the OECD Working Group would support the document.

B. Good Cell Culture Practices

Dr. Hartung, ECVAM, focused his presentation on good cell culture practices and the importance of the validation of *in vitro* methods to their use as alternatives. Dr. Hartung said discussion of this issue occurred at the 1999 Third World Congress on Alternatives and Animal Use in the Life Sciences. At that meeting, the General Assembly supported adoption of the *Bologna Declaration towards Good Cell Culture Practices* that called for development of guidelines defining minimum standards in cell and tissue culture. Dr. Hartung said the guidelines would be called Good Cell Culture Practice (GCCP), and their goal would be to promote international harmonization and standardization of laboratory practices, nomenclature, quality control systems, reporting, and safety procedures, and, where appropriate, to link to the application of GLP. The General Assembly hoped that these standards would be adopted by scientific journals in reviewing manuscripts for publication.

Dr. Hartung said he convened an ECVAM task force for development of the GCCP standards and the draft report was provided in the meeting materials for SACATM. He cited some reasons for needing GCCP in addition to GLP: 1) the inherent variation of *in vitro* test systems calls for standardization and defining optimal cell culture systems, 2) GLP gives only limited guidance for *in vitro* test systems, 3) GLP cannot be easily implemented in academia and 4) it would provide guidance for journals and funding groups. He said the task force report on GCCP (Hartung *et al.*, ATLA 30: 407-414, 2002) served as a resource for the draft OECD guidance document for *in vitro* toxicology studies. The task force planned a workshop in 2004 to discuss the final GCCP document with stakeholder groups, including cell culture societies, journals, and funding bodies. He suggested that this workshop might be a joint ICCVAM/ECVAM initiative.

Public Comments
None

Discussion
In response to a question from Dr. Hayes about support for the GCCP document, Dr. Schechtman reiterated that some OECD Working Group members felt that existing GLP principles are sufficient; however, the members' opinions were changed when they recognized the strong international support for guidance and the limitations of applying the current principles to new *in vitro* technologies. Dr. Green asked for clarification about exempting validation studies from following GLPs. Dr. Curren clarified that individual studies within a laboratory are conducted under GLP, but the design of a validation study is not governed under GLP. Dr. Hartung agreed and said this is an issue that needs to be addressed either through ICCVAM and ECVAM or OECD. Dr. Goldberg also sought clarification about GCCP versus GLP guidelines. Dr. Dean said the issue for GLP seems to be centered on developing guidance for the validation protocol as opposed to standards for cell culture practices used by individual laboratories. Dr. Hartung agreed and said the GCCP guidance would be directed toward the scientists who perform the *in vitro* studies; therefore, they want support for GCCP guidance from scientific journals and funding sources.

Dr. Portier asked the SACATM to address the questions posed in their meeting materials, and Dr. Dean asked Dr. Curren to lead the discussion. First, Dr. Curren said the SACATM would address GCCP and asked Dr. Acosta for his comments. Dr. Acosta supported the GCCP document as guidance to the scientific community. Dr. Curren felt the document addresses most issues and would be useful. Dr. Hayes said he is a journal editor and questioned how a journal would be assured that GCCP would actually be followed. Dr. Curren replied that the journal might require that the manuscript state whether GCCP standards were followed. Dr. Hayes felt that the scientific community would self-regulate cell culture practices without CGGP guidelines based upon whether the results are reproducible by other laboratories.

Dr. Curren asked whether the SACATM members could suggest additional key points that should be addressed in the GCCPs and none were offered. Dr. Sonneschein noted that some issues identified in the GCCP guidance are outside of the investigator's control, such as the composition of reagents. Dr. Curren acknowledged this to be true and said the GCCP would require the investigator to monitor and document what is used and report it in the manuscript. Dr. Portier said the NIEHS seeks the SACATM's scientific opinion whether a GCCP document would be useful and scientifically what areas are important to include. He said it appeared that SACATM is supportive of GCCP guidance. He asked SACATM to rank order the elements in the guidance and identify any that should be excluded or that are missing.

The SACATM felt that it was not possible to address Dr. Portier's question about ranking ordering the elements, because the current document is only a proposal and does not include specifics. Dr. Acosta said the Society for *In Vitro* Biology (previously called the Tissue Culture Association) had provided guidance on terminology and definitions in this field. He recommended that documents prepared by this group and other similar groups be examined to gain insight about information that should be included in the GCCP document. Dr. Snyder suggested that the National Academy of Science (NAS) be commissioned to prepare the GCCP document, since they are divested from the participating organizations and represent the scientific community. Dr. Willhite disagreed.

Dr. Hartung clarified that the task force report is not a guidance document, but provides perspective on what should be included in a guidance document. He said the next step in the process would be to hold a workshop to gain input from stakeholders and welcomed it being an international effort.

Dr. Goldberg suggested that discussions of the GCCP guidance and GLP for *in vitro* methods be separated. He pointed out that the GCCP initiative is an ECVAM activity and supported it continuing. He felt that there is insufficient information for him to comment on whether there should be a document addressing GLPs for *in vitro* methods separate from the OECD's current GLP guidelines. Dr. Stokes commented that what is proposed is not separate GLP regulations for *in vitro* tesing, but a consensus document at the OECD level that would provide supplementary guidance about meeting the regulations for GLPs. He added that the FDA and EPA representatives to OECD are supportive of this initiative.

By unanimous acclimation, the SACATM supported the idea that any study conducted for validation be done under GLP, whether *in vitro* or *in vivo*. Based upon the discussion, Dr. Dean said SACATM recommended further refinement of the GCCP guidance document with attention given to reviewing existing documents that address these issues and compiling the information.

Dr. Curren provided some insight into why GLP guidance for *in vitro* methodologies is needed. He said his experience in setting up validation studies and training naïve laboratories about GLP had identified problems with applying GLPs for *in vivo* methods to *in vitro* methods. He cited as an example that GLP for *in vivo* methods requires a separate animal room for each study, and noted that it is hard to apply this requirement for tissue culture – does it mean a separate incubator or a separate chamber in the incubator?

Dr. Dean asked the lead discussants for this topic, Drs. Curren, Stitzel and Safe, to review the proposal for GLPs for *in vitro* methods and provide comment back to SACATM about what elements should be deleted and which ones should be added. Dr. Curren noted that Dr. Stitzel, who was absent, had concerns that the GLP document be broad enough so that a separate document for each type of method would not be required.

Dr. Dean asked for the SACATM's views on the third question – "As *in vitro* science moves towards routine use of genetically manipulated cells, are there special requirements needed in these guidelines to ensure that the cells are responding as expected (*e.g.,* positive controls, sequence analysis, gene expression, finger printing, etc.)?" Dr. Safe felt that this question was broad and could only be answered for specific situations. He thought the GLP document should mention it, but only in a general way. He said the GCCP document addresses this issue, but noted that it might need to be expanded to cover modern genomic and proteomic technologies. Based upon comments by the SACATM, Dr. Dean thought that the committee supported the guidance document including a general statement addressing, but that it would be premature to set guidelines. It was clarified for the SACATM that the GLPs for *in vitro* methodologies would be a consensus document prepared as an addendum to the original GLP principles.

Dr. Portier summarized the opinion provided by SACATM: 1) NIEHS, NIH and other agencies should look toward a guidance document for *in vitro* assays that would have scientific validity and acceptance. 2) *In vitro* validation studies for regulatory purposes should be conducted

under GLP. He added that the definition of GLP for those studies is not yet defined and the agencies would need to prepare the document and bring it back to the SACATM for comment.

Dr. Schechtman said the intent is to have the GLPs for *in vitro* methods adopted internationally. Dr. Portier replied that this is a separate issue for the agencies and not an issue for SACATM; however, Dr. Dean said SACATM would support it as an international effort.

VII. Minimum Performance Standards for *In Vitro* Corrosivity Test Methods

For a brief period, Dr. Acosta served as chair in Dr. Dean's absence. Dr. Stokes, NIEHS, provided a historical perspective on development of minimum performance standards (MPS) for *in vitro* corrosivity test methods. The EPA asked ICCVAM to develop MPSs for proprietary and non-proprietary *in vitro* corrosivity methods that would provide test method developers with a benchmark for acceptable performance. He said the ICCVAM accepted the request and developed definitions, the elements of the MPS, and a process for establishing them. The purpose of MPS is to provide a basis for evaluating the acceptability of proposed test methods that are mechanistically and functionally similar to a validated and accepted reference test method. Dr. Stokes identified the components of MPS:

- Minimum procedural standards – the essential structural, functional, and procedural elements of a validated test method that should be included in the standardized protocol of proposed mechanistically and functionally similar test methods. Any deviations from the MPS must be justified scientifically.
- Minimum list of reference chemicals – a subset of selected substances that have been used to evaluate the validated test method. The list needs to be representative of the chemical and product classes for which the validated test method is applicable and the range of responses that the validated method is capable of measuring or predicting. This would include substances giving negative responses as well as weak to strong. Any deviations from the MPS must be justified scientifically.
- Comparable levels of accuracy and reliability that should be achieved by the proposed test method when evaluated with the reference chemicals.

Dr. Stokes noted that although the MPS for the *in vitro* corrosivity test methods were developed retrospectively, in the future it would be done prospectively when the test method comes to ICCVAM for evaluation. Dr. Stokes briefly summarized the process for development of MPS.

- NICEATM and the ICCVAM working group would develop proposed MPS for each test method.
- An independent peer review panel would review the test method and proposed MPS and NICEATM would invite public comment through a Federal Register notice.
- After considering the public comments and panel's recommendations, NICEATM and the ICCVAM working group would prepare final MPS for submission to ICCVAM.
- ICCVAM would review and approve the final MPS. The MPS would be part of the ICCVAM evaluation report on the test method.
- The ICCVAM report would be transmitted to appropriate federal agencies and made available to the public.
- The MPS would be used to create generic test guidelines, such as those needed by OECD, since they do not accept guidelines for proprietary methods.

Dr. Stokes outlined the timeline followed for development of the MPS for *in vitro* corrosivity test methods. He said NICEATM worked with the ICCVAM Dermal Corrosivity and Irritation Working

Group to draft the MPS and they were presented to the ICCVAM in June. He noted that the review process is currently ongoing. Once the final MPS are approved by ICCVAM, they will be published as addendum to the four existing ICCVAM test method evaluation reports and made available to the public and federal agencies. He identified Amy Jacobs from FDA and Karen Hamernik from EPA as co-chairs of the ICCVAM Dermal Corrosivity and Irritation Working Group.

Public Comments

Sara Amundson, Doris Day Animal League, addressed the SACATM. Ms. Amundson raised concern about the tiered testing approach for Corrositex® and its codification through the MPS. She noted concern that the decision by the peer review panel for this as a stand-alone test is not followed specifically in the ICCVAM recommendation.

In response to the public comments, Dr. Stokes said the panel recommended incorporating Corrositex® into the tiered testing strategy, which has been adopted internationally and therefore considered by the EPA and CPSC programs. A positive response in this test can lead to classifying and labeling the chemical as a corrosive hazard. He pointed out that the testing strategy is for both corrosion and dermal irritancy, and if there is no corrosive response, then the next step is to evaluate whether the chemical is a dermal irritant. This additional testing would be done because the *in vitro* method can yield significant false negatives. Dr. Stokes said the *in vivo* test is initially conducted on one animal and if the outcome is positive, the chemical is labeled as a corrosive hazard and testing stops. If the response is dermal irritation, then testing is repeated sequentially with 2 more animals. He said ICCVAM recommended Corrositex® as a stand-alone method in certain situations where only corrosivity is being determined. Dr. Cushmac, Department of Transportation, said the agency has a regulation to test for skin corrosion. Corrositex® is accepted as an exemption to the regulation, but has not been formally adopted as a regulation by DOT.

Discussion

Dr. Stephens asked if U.S. agencies accept Corrositex® as a stand-alone test if the only endpoint of concern is skin corrosion. Dr. Stokes replied that it would be a policy decision of the individual federal agencies based on their statutory testing responsibilities and requirements and not a decision by the ICCVAM; ICCVAM only makes test method recommendations. Dr. Stephens asked about the guidance that ICCVAM provided on the use of this test method. Dr. Stokes replied that the ICCVAM recommended that Corrositex® might be appropriate as a stand-alone in certain circumstances and listed DOT as the only known example, but noted that there might be other situations. Dr. Hartung commented that he believes that the policy of only accepting positive results from *in vitro* tests hampers and limits the development and implementation of *in vitro* tests as replacements for testing in animals.

Dr. Hayes asked whether the MPS is a short cut to validation and Dr. Stokes replied yes. Dr. Hayes expressed concern that this strategy might discourage test method developers from coming forth with new alternative tests because developing a method that mimics an existing one would be cheaper than developing and validating a new one. However, overall he was supportive of MPS because it would allow small and medium-sized companies to get their products to market quicker and more cheaply. It was noted that while companies might wait until the MPS is in place, the test might be improved over the one for which the MPS was originally developed. Dr. Merenda, EPA, clarified that the EPA cannot require the use of a

method that is proprietary; therefore, the agency must have a set of performance-based standards that if met by Corrositex® or another method would allow use of the assay's data. He added that it is critical for MPS to exist for Corrositex® so that the method is acceptable from a regulatory perspective. He said EPA appreciates the effort put forth by ICCVAM in developing MPS because it will allow use of several proprietary methods such as Corrositex®, Episkin™ and Epiderm™.

Dr. Dean asked Dr. Acosta, the lead discussant for this topic, to begin discussion of the assigned questions. Dr. Acosta asked whether SACATM felt that MPS might be needed. Dr. Curren said he is in favor of not having to run a large-scale validation study for a new method for the same endpoint if the method performs with the MPS like the originally validated method; however, he had concern that some additional data about the assay's performance for chemicals other than the reference group might be needed. He noted that potentially a new method could be developed that works well on the reference chemicals used in the first validation study, but might not perform well if tested using a broader range of chemicals. Dr. Stokes agreed with this point and said that it might be important that all available data for the method be submitted for evaluation, since it would not likely go through a formal ICCVAM review. Dr. Portier asked Dr. Curren to elaborate on what additional data might be needed beyond performance with the reference chemicals. Dr. Curren replied that he would want mechanistic information about how the assay works and data on chemicals other than the reference group. Dr. Hartung agreed that performance of the assay should be demonstrated beyond the test set of chemicals. In response to a question from Dr. Stephens, Dr. Stokes clarified that MPSs would be developed for any adequately validated *in vitro* or alternative method. Dr. Green asked whether the MPSs could be applied to existing test methods not validated by ICCVAM standards. Dr. Stokes said that ICCVAM could evaluate the validation status of an existing method and recommend MPSs based upon its performance if the method were accepted by the regulatory agencies.

Dr. Acosta summarized the concept of MPSs. Using Corrositex® as an example, he said ICCVAM would develop MPS, and if a new test method met the MPS, then that method would be accepted as an alternative. Dr. Dean noted that the goal of MPS would be to promote development of generic test systems based upon the proprietary method Corrositex®, since the regulatory agencies cannot require a proprietary test. The SACATM accepted the concept of MPS, but wanted any new test to provide data on more than the reference set of chemicals.

Dr. Portier said the ICCVAM would develop MPS for other alternatives, but use a general structure for setting them up. Using Corrositex® as the example, he asked the SACATM if they had concerns with 1) the set of 40 reference chemicals and 2) validating a new method against the reference set of chemicals and not the database used to validate Corrositex®, which included 169 chemicals tested in animals.

Dr. Hayes questioned why a new test would have to meet more rigorous standards than the original test and require submitting data on more than the reference 40 chemicals. He said the new test should be validated against Corrositex®, not the original data for Corrositex®. Dr. Curren disagreed and thought the best database to validate a new method against would be the original animal database used to validate Corrositex®.

Dr. Smith asked whether the issue of MPS should be applied initially to only patented methods. Dr. Goldberg said the process should have flexibility and allow the test method developer to submit data on how the new method performs with the reference set as well as other chemicals. Dr. Curren replied thought that if the new method were not a "me too test," but were proposed as a replacement for the standard method, then it should not be evaluated using the MPS paradigm, but should be evaluated using the ICCVAM validation standards. Dr. Stokes agreed and said the MPS is for tests proposed for exactly the same purpose as the reference test that they're based on. The accuracy and reliability of the test should be comparable to the original test that it's being compared against.

Dr. Tice explained the approach taken in developing the MPS for "me too" assays and said it is based upon an expedited approach used by ECVAM in evaluating Epiderm™ versus the validated method Episkin™. In principle, if the performance characteristics - accuracy and reliability - of the "me too" assay are the same as the original assay, then it would be accepted. The test method developer would have to substantiate that the "me too" assay is not mechanistically or functionally different from the original test. However, if the intended purpose of the new test were expanded or different than the original test, then the new method would have to go through the full ICCVAM validation process. He said NICEATM used the standard method to establish criteria for selecting the chemicals in the reference set.

Dr. Dean asked Dr. Acosta, with assistance from Dr. Monteiro-Riviere (absent from the meeting), to prepare a response to the questions and it would be provided to the SACATM at its next meeting.

VIII. ICCVAM/NICEATM Expert Panel Meeting on *In Vitro* ER/AR Assays

Dr. George Daston, Procter and Gamble Company, provided the SACATM with information about an ICCVAM-NICEATM expert panel meeting that he chaired to evaluate the validation status of *in vitro* estrogen receptor and androgen receptor and transcriptional activation assays. This presentation was originally scheduled for the December 2002 meeting, but cancelled because Dr. Daston was unable to attend the meeting because of bad weather.

He provided an historical perspective about events leading up to the expert panel meeting. In 1996, two laws were passed requiring the EPA to evaluate the estrogenic and other endocrine disrupting properties of pesticides used in foods and of drinking water contaminants. EDSTAC, a federally chartered advisory committee of the EPA, recommended that the evaluation also include an assessment of the androgenic and thyroid disrupting properties of chemicals. EDSTAC proposed a tiered approach to prioritize, screen and test the chemicals for biological activities and a timetable for implementation of the program. As part of this screening program, EPA needed a standardized approach for assessing estrogen receptor and androgen receptor interactions. EPA asked the ICCVAM to evaluate the validation status of these types of assays. ICCVAM agreed and asked NICEATM to convene an expert panel peer review.

NICEATM prepared comprehensive background documents on each of the four types of assays using contract support. The panel was charged with providing recommendations about which assays should be considered for future validation and their relative priority, the adequacy of the proposed minimum procedural standards for each of the four types of assays, the adequacy of the protocols for assays recommended for validation studies, and the adequacy of the test substances recommended for the validation studies. Dr. Daston identified the panel and

acknowledged Dr. Safe's participation and recognized the chairs of the working groups. Dr. Daston said the expert panel concluded that there is no standardized, validated protocol for any of the methods; however, the panel was able to establish some minimum procedural standards for them.

Dr. Daston briefly summarized the panel's recommendations. The panel recommended that highest priority be given to development of estrogen receptor binding assays that use purified recombinant protein, because it would identify individual isoforms of the receptors, eliminate the need for tissue preparation and standardization, reduce animal use, and enable testing of the human receptor. Dr. Daston noted that the assay system, if possible, should include a method for exogenous metabolic activation and should take advantage of nonradioactive ligands. The panel recommended that the rat uterine cytosol protocol be revised to incorporate the minimum procedural standards and then used as a template for other assays. Dr. Daston said the background review document recommended 33 substances. The panel modified the list of substances for testing in the validation studies of the estrogen receptor binding assays by increasing the number of nonestrogenic substances and recommended that the same substances be tested in both the estrogen receptor binding and transcriptional activation assays.

The panel concluded that the data available from the estrogen receptor transcriptional activation assays were not adequate for their evaluation and no specific assay was recommended. Dr. Daston said the panel recommended a prevalidation study to compare stably transfected and transiently transfected cell lines, but did not recommend development of an exogenous metabolic activation system. He said standardized procedures for assay set-up and assay protocols would need to be developed. The standardized assays would be tested with a minimum set of 52 substances.

Next, Dr. Daston discussed the androgen receptor binding assays and said no standardized, acceptable protocol existed, but the panel thought that the rat prostate cytosol assay might be adapted if augmented with the minimum procedural standards. The panel recommended that an assay using purified recombinant androgen receptor be developed; however, legal issues of a licensing agreement must be worked out because the human androgen receptor is patented. He said alternatively, a recombinant androgen receptor from another primate species might be used.

Finally, Dr. Daston noted that only a small number of transcriptional activation assays exist for the androgen receptor and none was acceptable to the panel. The panel recommended development of an assay that would use cells containing an endogenous androgen receptor stably transfected with an adenovirus containing MMTV-Luc reporter gene. The minimum validation set would include 53 compounds.

The panel did not recommend exogenous metabolic activation for the assays in order to expedite the standardization and validation of their protocols. The panel did recommend a central repository of coded chemicals for the validation studies and suggested that the EPA consider a sequential testing strategy. In closing, Dr. Daston said the protocols for the binding assays need to be standardized and validated. The transcriptional activation assays are less well developed and have many technical challenges to overcome before they can be standardized and validated.

Public comments

Dr. Errol Zeiger, consultant from Chapel Hill, said he consulted for ILS at the time of the expert panel meeting and attended the meeting. He expressed concern about the criteria used for determining the number of chemicals to be used in the validation assays and for selecting the reference chemicals, especially those to be used as negative controls. He felt in many incidences that the data available for developing the list of chemicals were inadequate. He stressed the importance of having sufficient data available to support selecting chemicals for a reference list.

Dr. Dean acknowledged receipt of written comments from Drs. Rosen, Marschke, and Negro-Vilard from Ligand Pharmaceuticals. The comments were distributed to the SACATM prior to the meeting.

Discussion

Dr. Dean asked Drs. Wilhite and Sonneschein, lead discussions for this topic, to begin the committee's discussion. Dr. Willhite commended the expert panel for its comprehensive and careful analysis of the assays and acknowledged that the four background documents prepared by NICEATM for this review were well organized and obviously integral to the quality of the panel's final report. He felt that the panel was somewhat limited in the availability of information and its charge. *For example*, Dr. Willhite said the Japanese Ministry of Health's protocols were not included in the panel's data sets and the panel was not asked to consider gene expression patterns when evaluating receptor binding outcomes. He raised questions about species selection for the recombinant receptor and noted potential problems of biotransformation. He suggested that the definition and criteria for assessing *in vitro* cytotoxicity needed to be carefully delineated as well as the statistical criteria for judging the assays' results relative to the historical controls. He also stated that it was important to haven high quality in vitro data available for developing quantitative structural activity relationships (QSAR).

Dr. Daston responded that the transcriptional activation assays would assess gene response and the panel recommended that genomic approaches be considered as the assays evolve. He agreed that choosing which species' receptor to use is an important issue. The panel recommended using a human recombinant receptor; however, the panel said it would be an EPA policy decision with regard to evaluating the impact of endocrine disrupting agents on other mammalian and non-mammalian species. The minimum procedural standards are amenable to the use of other kinds of receptors. In terms of cytotoxicity, the panel focused on identifying minimum procedural standards specific for estrogen and androgen transcriptional assays. He acknowledged that many methods exist for evaluating cytotoxicity and the final assay used in the validation study needs to have specific guidance on this issue. Dr. Daston said the panel's recommendations do address data needs for an acceptable test. He believes the data from the binding assays will serve as a foundation for further refinement of the assays and development of QSARs.

Dr. Portier asked the SACATM to consider whether an *in vitro* assay is needed if molecular modeling of the receptor-binding region were developed. Dr. Willhite noted that conceptually QSAR is appealing; however, it can be misleading and yield results inconsistent with biology. He said it is a more useful tool for confirming assay findings. Dr. Sonneschein raised concern about relying upon *in vitro* as opposed to cell culture assays for screening estrogenic

compounds since historically estrogen mimics were identified by bioassays, not binding assays. Dr. Daston replied that EPA is relying on the EDSTAC's recommendations to limit the screening battery to *in vitro* receptor binding and transcriptional activation assays. He noted the panel's charge was to examine these types of assays. He added that the EDSTAC had concern that the cell culture bioassays are less specific than the receptor binding and transcriptional activation assays. Dr. Sonneschein noted a publication in *Environmental Health Perspectives* by Andersson and coworkers that compared the transcriptional assay, binding assay and the E-screen bioassay and concluded that the E-screen is the most reliable.

Dr. Dean allowed Dr. Zeiger to provide additional details about the background information for the expert panel meeting. Dr. Zeiger said the data from the bioassays identified by Dr. Sonneschein were summarized as part of the background documents. Based upon his knowledge of this topic, Dr. Sonneschein felt that the bioassay is better than the assays the panel was asked to evaluate. Dr. Safe said the panel's charge did not include assessing the suitability of assays other than the receptor binding and transcriptional activation assays. Dr. Daston acknowledged that he too had some dissatisfaction with the assays posed to the panel for evaluation.

Dr. Schrader raised several points that he considered could impact an evaluation of the endocrine disrupting activity of chemicals: 1) defining a compound as an agonist or antagonist depends upon the tissue and the organism, 2) sequence differences in the androgen receptor are common, and 3) ligand independent activation of these receptors can occur.

IX. General Discussion

Dr. Dean said that because of time constraints, discussion of agenda item on transgenics would be postponed until the next meeting. Since, Dr. Richard Becker had asked to make public comment on this topic, Dr. Dean invited him to make comments now or wait until the next meeting. Dr. Becker chose to make some brief comments.

Public Comments

Dr. Richard Becker from the American Chemistry Council (ACC), asked the SACATM to review the comments submitted by the ACC. He said transgenic animal models have undergone significant development and standardization and the NTP is using them and reporting the results in a technical report series. He said there is a lack of consensus about the models' relevance and reliability. He said they should be reviewed using the ICCVAM process as a way to achieve a consensus of understanding across the 15 federal agencies within ICCVAM.

Information on Alternatives

Dr. Acosta made some general comments about the topics that had been covered. He said the agencies were involved in similar activities (e.g., toxicogenomics) and should communicate among themselves to prevent redundancy of effort, including maintenance of databases, and enhance cooperation. He reiterated SACATM's need to have a better understanding of the resources being spent on alternatives by the agencies. Dr. Acosta said the information Dr. Portier provided on alternatives from NIEHS grants is helpful, but asked that more specific information be provided on resources for individual components (*e.g., use of alternatives* versus *development* versus *validation*). He supported NIEHS sponsoring another RFA on alternatives

like the one done previously. He said SACATM needs a better understanding of NIEHS priorities and resources for alternatives and asked about the budget for ICCVAM and NICEATM.

Dr. Dean asked the SACATM to consider several issues:
- Proposals presented by the agencies where validation of alternative methods is needed
- A subcommittee to address gaps in information about alternative and set priorities
- A subcommittee to develop a strategic plan for SACATM
- Opportunities where alternative methods would greatly impact the 3Rs

Drs. Willhite and Stephens emphasized the need for information from the agencies that includes both internal and external activities on alternatives, the budget and identifiable targets to enable SACATM to understand where government-wide needs might be met and help set priorities.

Dr. Kulpa-Eddy, USDA, elaborated on her agency's need saying that the *in vitro* test needs to validated against the *in vivo* test in the host animal. The USDA is focused on replacing the *in vivo* vaccine challenge tests used for determining potency, because they cause pain and distress to animals. Dr. Goldberg pointed out that Europe has considerable activity to develop these type of methods that is being led by Dr. Coenraad Hendriksen from the Netherlands and Dr. Claus Cussler from Germany. Dr. Hartung added that Dr. Hendriksen and Cussler are involved on an ECVAM task force and ECVAM will help sponsor a future workshop with the European Pharmacopoeia on biologics. He supported the workshop being a joint effort between ICCVAM and ECVAM.

Dr. Goldberg recommended that a unified approach with ECVAM be developed to address validation of *in vitro* methods that would replace the *in vivo* vaccines. Dr. Portier said an agenda topic for the next SACATM meeting would be an update on the USDA project and funding for the validation.

Dr. Hayes asked for information at the next meeting about what efforts are underway for development of vaccine alternatives for humans. Dr. Schechtman said he would look into having a representative from the FDA Center for Biologics Evaluation and Research speak on this issue at the next SACATM meeting. Dr. Hayes asked for information from the Department of Defense (DoD) about efforts on alternative vaccines. Ms. Decot from DoD said development of alternative vaccines is an area where animal use could be reduced that DoD identified in the ICCVAM survey. She said information about DoD's expenditures on animal research and its priority areas is available on the Internet at http://www.dtic.mil/biosys. Also on-line is a report about annual animal use and a link to a DoD database on intramural and extramural funding.

The SACATM agreed that the committee would like to learn more about the status and opportunities for alternatives to vaccines and asked the ICCVAM representatives to identify how this area might be improved.

Ocular Toxicity
The SACATM next discussed ocular toxicity. Dr. Willhite supported moving forward on learning more about needs in ocular toxicity testing. Dr. Curren said this is an important area for the consumer products and cosmetic industries because they do not want to do the rabbit eye test for safety testing. Dr. Hayes suggested that ICCVAM might sponsor a workshop to find out what alternatives are available, the status of their validation and how this area might move

forward. Dr. Stokes said the Interagency Regulatory Alternatives Group (IRAG) held a workshop about 10 years ago and Dr. Green was a member. He said a workshop could be held to identify the available methods, their validation status, and the research and development needs; this is the mechanism ICCVAM followed for its review of *in vitro* methods for acute toxicity.

Dr. Green said ocular toxicity testing needs addressing; however, past efforts have been unsuccessful in moving from development of good methods to their application. As a starting point, he suggested that the IRAG reports be reviewed and the status of their recommendations be evaluated. In response to a question from Dr. Willhite about the time and effort required for preparing a background document on ocular toxicity testing, Dr. Stokes said the contract support for NICEATM would enable it to move forward if funding were available. Dr. Portier did not agree with moving forward on this specific task at this time. He felt that the SACATM's initial plans to have ICCVAM provide information about where animals are being used and what tests cause pain and distress, etc., would guide the committee in making recommendations on priorities. He acknowledged that ocular toxicity might be a priority; however, Dr. Portier said he would not move on the committee's recommendation for ocular toxicity until a more complete evaluation of the agencies' needs for alternatives is completed. Dr. Schechtman said that ICCVAM surveyed its member agencies and ocular toxicity is the top priority.

Dr. Hayes made a motion: The SACATM recommends that an evaluation of ocular toxicity be conducted that includes having NICEATM prepare a background document and, if appropriate, hold a workshop. Dr. Willhite seconded the motion. The motion carried with 10 yes votes and 2 abstentions (Goldberg and Curren). Goldberg and Curren abstained from voting due to conflicts of interest. Dr. Goldberg suggested that companies involved in the development of tests for ocular toxicity be involved in any evaluation and Dr. Curren suggested including ECVAM to prevent duplication of efforts on this topic.

Dr. Green said that while he is concerned about ocular toxicity testing, he understood Dr. Portier's reluctance to move forward with the recommendation on ocular toxicity without having information about other needs for alternatives to help set priorities for available resources. He supported ICCVAM assembling information about each agency's needs for alternatives and suggested using a common format that includes cost, a rationale for the need and why the need should be a priority. He said this information could be used in an overall evaluation to guide the SACATM in making recommendations for priorities.

Dr. Stokes said that ICCVAM would expand its survey of the agencies on areas of need for alternative test methods and ask that a rationale be given for each need. He would provide the results to the SACATM at the next meeting. Dr. Hayes asked if part of the survey to the regulatory agencies could include questions about animal use. He suggested that the agencies provide for each required test method information about the animal models being used, the average yearly animal usage to meet the test requirement, and whether alternative test methods are available. Dr. Stokes said in some areas, such as pesticides, there would be data submitted by industry to the EPA; however, in other areas, such as cosmetics and consumer products, the data are not provided to the agencies. Dr. Portier said that NIEHS would supply the requested information. In order to get the information from each agency, Dr. Portier said the best process would be for ICCVAM to develop a questionnaire. Dr. Olden would then send the questionnaire to the agencies' executive secretaries who could canvass the entire agency. Dr. Portier agreed

to inform Dr. Olden of SACATM's request for information about animal use and to ask if he would agree to send a questionnaire. Dr. Portier acknowledged that it would be each agency's decision whether to respond to Dr. Olden's request. Dr. Portier said he would follow-up on this issue with SACATM at the next meeting.

Dr. Smith acknowledged the value of having Dr. Hartung at the SACATM meeting and encouraged international collaboration on validation efforts to promote global use of alternatives and the judicious use of animals, resources, and data.

Agency Expectations of SACATM
Dr. Dean asked the ICCVAM agency representatives to define their expectations of SACATM.

Dr. Joe Merenda from EPA said the agency would benefit from SACATM's advice on priorities and directions for the development and validation of alternative methods across the federal government. He also felt that the scientific advice, experience of the members, and the breadth of perspective from a committee specifically focused on alternative methods would be beneficial to the EPA in helping identify opportunities and ideas and in understanding the range of issues.

Dr. Schechtman from FDA said the agency would look to the SACATM for advice and direction for improving processes, priorities, productivity and resources, both financial and personnel. He said areas where help with resources would be needed include funding for both methods validation and to fund ICCVAM collaborations with ECVAM. He said the advice from SACATM should address science and policy.

Dr. Portier from NIEHS noted the changing face of toxicology as science advances. He said he looks to SACATM to provide direction and help set priorities for using new alternative methods in toxicological testing that take advantage of advances in science, such as new molecular and mechanistic-based methods, and for preparing regulatory toxicology for the future.

Ms. Patty Decot, DoD, said her agency, which is nonregulatory, has no expectations or tasks for SACATM, but welcomes the opportunity to collaborate, leverage resources and take advantage of SACATM's advice and apply it to research within DoD to make the program stronger.

Dr. Stokes said he looks to SACATM to provide advice on priorities, directions and processes applicable to the mission of NICEATM and ICCVAM.

SACATM Subcommittees
Dr. Dean proposed that the SACATM form a subcommittee to develop a strategic plan that incorporates the expectations of the agencies and that can guide the committee in achieving some of those objectives. He would hope the plan will outline the committee's mission, what the needs are in terms of that mission, and what the SACATM views as priorities. He would envision the plan being action-oriented. Dr. Portier said SACATM has a charter outlining the committee's purpose and objectives and a copy would be sent to the members. He said the charter could be refined, but not changed until at the time of renewal.

Dr. Dean proposed a second subcommittee to review gaps and needs for alternatives and set priorities for them. He supported Dr. Hayes' suggestion to obtain information from the agencies about the areas where having alternative methods would make the greatest impact on animal

use and the 3Rs. Dr. Dean asked members to provide their names to Dr. Wolfe. Dr. Sonneschein suggested that endocrine disruptors might be an area to explore. Dr. Portier replied that it might be of low priority until the EPA provides the SACATM with a full review of the status of testing in this area. Dr. Dean agreed.

Dr. Curren felt that information about animal use for regulatory activities is also needed from industry. Dr. Schechtman supported this suggestion of involving industry to get greater accuracy about animal use. He said industry and contract laboratories conduct the research and testing submitted to FDA and the agency is not knowledgeable about the full extent of animal use, because failed tests are not reported and industry is not obliged to submit data on animal use for products during their developmental stages. Dr. Dean noted that getting this information might be quite difficult. Dr. Hayes said Gillette and Colgate used no animals for regulatory purposes in the past two years and this information is published. Dr. Willhite asked whether trade associations might also provide this information. Dr. Dean said the pharmaceutical industry turns in information about use of control animals for regulated species to the USDA regularly, but that data does not include use of rodents. Dr. Curren noted that NIH is also a large user of animals, but this is not for regulatory purposes.

Dr. Goldberg asked whether the strategy suggested by Dr. Portier to have Dr. Olden contact the agencies' executive secretaries for information about where alternative methods would be useful and have the greatest impact on animal use might be extended to include industry groups, such as the American Chemistry Council and pharmaceutical manufacturers association. Dr. Portier thought this possible and suggested that the inquiry be two-fold: *first* where are animals used and in what capacity and *second* what are the areas where having alternatives would make the greatest impact on animal use. Dr. Goldberg accepted this approach.

Dr. Stokes noted that the USDA's annual report on animal use has information on regulated species, but this does not include mice, rats and birds. The information includes 1) procedures where unrelieved pain and distress are involved, 2) where pain and distress are treated with analgesics, anesthetics and sedatives, and 3) where there is no pain and distress. He said Dr. Stephens did a report several years ago that showed much (~70%) of the unrelieved pain and distress was associated with veterinary biologics testing. The animal use for other testing that involved unrelieved pain and distress included mainly guinea pigs for maximization tests of dermal sensitization and rabbits for ocular eye irritation and dermal irritation corrosivity. Dr. Theran questioned the utility of that information since covered species account for about 5% of total animal use, and therefore most animal use is not reported on the USDA forms.

Public comments

Jim Sherman, a toxicologist from BASF, addressed the SACATM. BASF is the world's largest chemical company. Dr. Sherman said he works for the Agricultural Products Division, the world's third largest agricultural products company. His first addressed BASF's efforts on alternative animal testing and cited some of the tests being used by BASF that include both validated and non-validated methods. He said BASF is involved in a validation study in Europe for a nonradioactive method based on the local lymph node assay. Second, he supported global acceptance and validation of alternative methods to help ensure international reduction in animal use. He felt SACATM should include international representation. He noted that the best way to reduce animal use is through good professional judgment and suggested that

guidance be provided on when a study is needed. Finally, he said one area where an alternative method would be beneficial to BASF would be for *in vitro* dermal penetration. He noted that rodents are not a good species for this testing, so either a primate or human study is required, which BASF would like to avoid. Finally, he stated that they routinely conduct an in vitro HET-CAM assay for ocular irritation prior to conducting the animal test.

Dr. Portier addressed the issue of international representation on SACATM. He noted that federal advisory committee rules for committees make it extremely difficult, essentially prohibitive, to having a non-U.S. citizen be a member.

Dr. Willhite suggested that Mr. Sherman's suggestion for an alternative test for *in vitro* dermal penetration be considered as the SACATM addresses areas for testing and priorities.

Dr. Hayes asked Dr. Stokes to update SACATM on the international status of tests for dermal penetration. Dr. Stokes said two OECD test guidelines were adopted, one for *in vivo* and one for *in vitro*. Most of the data generated to date are being used for benchmarking the *in vitro* results against the *in vivo* findings for reference chemicals. The need for careful standardization and validation of an *in vitro* dermal absorption method is well recognized.

Closing Remarks
Dr. Dean introduced a new procedure for SACATM. He asked that members assigned as lead discussants for a topic prepare a 1-2-page critique prior to the meeting that could serve as the basis for committee discussion. He also asked the persons assigned to topics for the current meeting prepare a similar critique and send to Dr. Wolfe for SACATM and the record.

Dr. Dean thanked all participants, the committee, Dr. Hartung and ICCVAM for participating in the meeting.

On behalf of Dr. Olden, Dr. Portier thanked everyone for their time and effort toward the discussions. He recognized staff contributions to the meeting. He said he would take the SACATM's recommendations to Dr. Olden and provide follow-up at the next meeting. He noted that NIEHS would make its best effort in considering and moving forward on SACATM's recommendations. He thanked participants, including SACATM, Dr. Hartung, ICCVAM, and Dr. Dean as chair. NIEHS will consider whether to hold the next meeting in North Carolina or Washington. Dr. Hayes asked if future meeting might be set and Dr. Wolfe said the staff would try, but in the past this has been difficult because of calendar uncertainties of members. The goal is for SACATM to meet biannually.

The meeting adjourned at 2:46 p.m.

Cancer Institute, National Cancer Institute, National Institutes Of Health, 6116 Executive Boulevard, Room 8057, MSC 8329, Bethesda, MD 20892–8329, 301–496–7421, *kerwinm@mail.nih.gov*.

This notice is being published less than 15 days prior to the meeting due to the timing limitations imposed by the review and funding cycle.

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

Dated: July 10, 2003.

Anna P. Snouffer,

Acting Director, Office of Federal Advisory Committee Policy.

[FR Doc. 03–18007 Filed 7–15–03; 8:45 am]

BILLING CODE 4140–01–M

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

National Cancer Institute; Notice of Closed Meeting

Pursuant to section 10(d) of the Federal Advisory Committee Act, as amended (5 U.S.C. Appendix 2), notice is hereby given of the following meeting.

The meeting will be closed to the public in accordance with the provisions set forth in sections 552b(c)(4) and 552b(c)(6), Title 5 U.S.C., as amended. The grant applications and the discussions could disclose confidential trade secrets or commercial property such as patentable material, and personal information concerning individuals associated with the grant applications, the disclosure of which would constitute a clearly unwarranted invasion of personal privacy.

Name of Committee: National Cancer Institute Special Emphasis Panel, Review of Administrative Supplement Applications for Disseminating Evidence-based Intervention Research Products.

Date: August 4, 2003.

Time: 8:30 a.m. to 5 p.m.

Agenda: To review and evaluate program documents.

Place: National Institutes of Health, 6130 Executive Blvd, Conference Room C, Rockville, MD 20852, (Telephone Conference Call).

Contact Person: Cynthia Vinson, MPA, Program Analyst, National Cancer Institute, Division of Cancer Control and Populations Sciences, 6130 Executive Blvd., Room 7046, Bethesda, MD 20892, 301/594–5906.

This notice is being published less than 15 days prior to the meeting due to the timing limitations imposed by the review and funding cycle.

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

Dated: July 10, 2003

Anna P. Snouffer,

Acting Director, Office of Federal Advisory Committee Policy.

[FR Doc. 03–18008 Filed 7–15–03; 8:45 am]

BILLING CODE 4140–01–M

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

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

Pursuant to section 10(a) of the Federal Advisory Committee Act, as amended (5 U.S.C. Appendix 2), notice is hereby given of a meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) on August 12–13, 2003, in the Rodbell Auditorium, Rall Building at the National Institute of Environmental Health Sciences, Research Triangle Park, NC. The meeting begins each day at 8:30 a.m.

Agenda

The meeting is being held on August 12–13, 2003 from 8:30 a.m. until adjournment and is open to the public with attendance limited only by the space available. Individuals who plan to attend are asked to register with the NTP Executive Secretary (NTP Liaison and Scientific Review Office, NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709; telephone: 919–541–0530; facsimile: 919–541–0295 or *wolfe@niehs.nih.gov*). The names of those registered will be given to the NIEHS Security Office in order to gain access to the campus. Persons attending who have not pre-registered may be asked to provide pertinent information about the meeting, *i.e.*, title or host of meeting before gaining access to the campus. All visitors (whether or not you are pre-registered) will need to be prepared to show 2 forms of identification (ID, *e.g.*, driver's license, government ID).

Persons needing special assistance, such as sign language interpretation or other reasonable accommodation in order to attend, are asked to notify the NTP Executive Secretary at least seven business days in advance of the meeting (see contact information above). Plans are underway for making this meeting available for viewing on the Internet (*http://www.niehs.nih.gov/external/video.htm*).

A preliminary agenda is provided below. A copy of the agenda, committee roster, and any additional information, when available, will be posted on the NTP Web site (*http://ntp-server.niehs.nih.gov*) or available upon request to the NTP Executive Secretary (contact information provided above). Following the meeting, summary minutes will be prepared and available through the NICEATM/ICCVAM Web site (*http://iccvam.niehs.nih.gov*) and upon request to the NTP Liaison and Scientific Review Office (contact information above).

Preliminary Agenda

Scientific Advisory Committee on Alternative Toxicological Methods August 12–13, 2003

Rodbell Auditorium, Rall Building, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC 27709

August 12, 2003

8:30 a.m.—Call to Order and Introductions
Welcome from the NIEHS Director
National Toxicology Program Update
Update on Activities of the NTP Center for the Evaluation of Alternative Toxicological Methods and the Interagency Coordinating Committee on the Validation of Alternative Methods
Update on Activities of the European Centre for the Validation of Alternative Methods
U.S. Federal Agency Efforts in Test Method Development and Validation
- Environmental Protection Agency
- National Center for Toxicological Research of the Food and Drug Administration

11:50 a.m.—Lunch Break (on your own)
1 p.m.
- U.S. Department of Agriculture
- National Institutes of Health, Office of the Director
- National Cancer Institute
- National Institute of Environmental Health Sciences and the NTP

Public Comments
5 p.m.—Adjourn

August 13, 2003

8:30 a.m.—Introductions and Call to Order

Application of GLPs to *In Vitro* Test Methods
- ICCVAM/ECVAM Proposal for Development of International Guidance
- ECVAM Guidelines for Good Cell Culture Practices
- Public Comments

Minimum Performance Standards for Test Methods
- MPS for In Vitro Corrosivity Methods
- Public Comments

In Vitro Endocrine Binding and Transcriptional Activation Assays: Minimum Procedural Standards and Reference Chemicals
- Public Comments

12:05 p.m.—Lunch (on your own)

1 p.m.—Overview of ILSI/HESI Work Group's Activities on Identification of Biomarkers of Toxicity and Summary of First Meeting

Validation of Genetically Modified Mouse Models
- Public Comments

2:45 p.m.—Adjourn

Public Comment Welcome

- Public input at this meeting is invited and time is set aside for the presentation of public comments on any agenda topic. Each organization is allowed one time slot per agenda topic. At least 7 minutes will be allotted to each speaker, and if time permits, may be extended to 10 minutes. In order to facilitate planning for this meeting, persons wishing to make an oral presentation are asked to notify the NTP Executive Secretary (contact information above) by August 4, 2003, and to provide their name, affiliation, mailing address, phone, fax, e-mail, and sponsoring organization (if any). Registration for oral comments will also be available on-site, although time allowed for presentation by on-site registrants may be less then that for pre-registered speakers and will be determined by the number of persons who register at the meeting.

Persons registering to make oral comments are asked, if possible, to provide a copy of their statement to the NTP Executive Secretary (contact information above) by August 4, 2003, to enable review by the SACATM and NIEHS/NTP staff prior to the meeting. Written statements can supplement and may expand the oral presentation. If registering on-site and reading from written text, please bring 40 copies of the statement for distribution to the SACATM and NIEHS/NTP staff and to supplement the record. Written comments received in response to this notice will be posted on the NTP Web site (*http://ntp-server.niehs.nih.gov*).

Persons may also submit written comments in lieu of making oral comments. Written comments should be sent to the NTP Executive Secretary and should be received by August 4, 2003, to enable review by the SACATM and NIEHS/NIH prior to the meeting. Persons submitting written comments should include their name, affiliation, mailing address, phone, fax, e-mail, and sponsoring organization (if any) with the document.

Background

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

Dated: July 9, 2003.

Samuel Wilson,

Deputy Director, National Institute of Environmental Health Sciences.

[FR Doc. 03–18012 Filed 7–15–03; 8:45 am]

BILLING CODE 4140–01–P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

National Toxicology Program (NTP); National Institute of Environmental Health Sciences (NIEHS); National Institutes of Health (NIH); NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) Request for Existing Dermal and Ocular Irritancy Chemical Test Data From Animal and Human Studies Using Standardized Testing Methods

Summary

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and NICEATM are collaborating with the European Centre for the Validation of Alternative Methods (ECVAM) to conduct a validation study on *in vitro* test methods for assessing dermal irritation. Future collaborative validation studies may evaluate alternative methods for assessing ocular irritancy or other hazard endpoints. On behalf of ICCVAM, the NICEATM requests the submission of existing data on commercially available chemicals tested for skin irritancy in rabbits using current standardized testing methods (*e.g.*, EPA 1998a; EPA 1998b; OECD 2001). These data will be used to help identify appropriate reference chemicals (*i.e.*, those with high-quality *in vivo* testing data) for use in the validation study. NICEATM welcomes the submission of existing data from both human and animal studies and is also interested in any human post-marketing or occupational exposure/surveillance data that might be available for these chemicals. NICEATM also requests the submission of existing, high quality ocular irritation data that might be used to identify appropriate reference chemicals for future validation studies of *in vitro* ocular irritancy test methods. Data are sought from studies conducted to comply with Federal or other national/ international testing requirements that may not be publicly available because, (1) it was submitted to regulatory authorities, but cannot be released to the public by regulatory authorities, or (2) there is no requirement to submit the data to regulatory authorities.

Request for Submission of Chemical and Protocol Information/Test Data

Data and other information submitted in response to this notice should be sent by mail, fax or e-mail to NICEATM [Dr. William S. Stokes, Director, NICEATM, NIEHS, PO Box 12233, MD EC–17, Research Triangle Park, NC, 27709, (phone) 919–541–2384, (fax) 919–541–0947, (e-mail) *iccvam@niehs.nih.gov*] by noon on September 2, 2003 in order to ensure their consideration for the upcoming *in vitro* dermal irritation validation study. However, data and information received after this date will be periodically compiled and added to the database maintained by NICEATM. All chemical and protocol information/ test data submitted in response to this notice will be publicly available upon request to NICEATM.

When submitting chemical and protocol information/test data, please reference this **Federal Register** notice and provide appropriate contact information (name, affiliation, mailing address, phone, fax, e-mail, and sponsoring organization, as applicable).

Tentative Agenda

Scientific Advisory Committee on Alternative Toxicological Methods
August 12-13, 2003
National Institute of Environmental Health Sciences

August 12, 2003

8:30 AM	**CALL TO ORDER AND INTRODUCTIONS**	Dr. Jack Dean, Sanofi-Synthelabo, Inc., Chair
8:45 AM	**WELCOME AND REMARKS FROM NIEHS DIRECTOR**	Dr. Kenneth Olden, NIH/NIEHS
9:00 AM	**NATIONAL TOXICOLOGY PROGRAM UPDATE**	Dr. Christopher Portier, NIH/NIEHS
9:15 AM	**UPDATE ON ACTIVITIES OF NTP INTERAGENCY CENTER FOR THE EVALUATION OF ALTERNATIVE TOXICOLOGICAL METHODS AND THE INTERAGENCY COORDINATING COMMITTEE ON THE VALIDATION OF ALTERNATIVE METHODS**	Dr. William Stokes, NIH/NIEHS
9:50 AM	**UPDATE ON ACTIVITIES OF THE EUROPEAN CENTRE FOR THE VALIDATION OF ALTERNATIVE METHODS**	Dr. Thomas Hartung, ECVAM
10:10 AM	***BREAK***	
10:30 AM	**U.S FEDERAL AGENCY EFFORTS IN TEST METHOD DEVELOPMENT AND VALIDATION**	
	· Environmental Protection Agency	Dr. Joseph Merenda, EPA
	· National Center for Toxicological Research of the Food and Drug Administration	Dr. Daniel Casciano, FDA/NCTR
11:50 AM	***LUNCH***	
1:00 PM	· U.S. Department of Agriculture	Dr. Jodie Kulpa-Eddy, USDA
	· National Institutes of Health/Office of the Director	Dr. Margaret Snyder, NIH/OD
	· National Cancer Institute of the National Institutes of Health	Dr. Alan Poland, NIH/NCI and Dr. Raju Kucherlapati, Harvard Center/Partners for Genetics and Genomics
3:00 PM	***BREAK***	
3:20 PM	· National Institute of Environmental Health Sciences of the National Institutes of Health and the NTP	Dr. Christopher Portier, NIH/NIEHS
4:00 PM	· Public Comments	
4:15 PM	· Committee Discussion	
5:00 PM	***ADJOURN***	

Tentative Agenda

Scientific Advisory Committee on Alternative Toxicological Methods
August 12-13, 2003
National Institute of Environmental Health Sciences

August 13, 2003

8:30 AM	**CALL TO ORDER AND INTRODUCTIONS**	Dr. Dean, Sanofi-Synthelabo, Inc., Chair
8:40 AM	**APPLICATION OF GLPS TO *IN VITRO* TEST METHODS** · ICCVAM/ECVAM Proposal for Development of International Guidance · ECVAM Guidelines for Good Cell Culture Practices · Public Comments · Committee Discussion	Dr. Leonard Schechtman, FDA/NCTR Dr. Thomas Hartung, ECVAM
9:55 AM	***BREAK***	
10:15 AM	**MINIMUM PERFORMANCE STANDARDS FOR *IN VITRO* CORROSIVITY TEST METHODS** · Public Comments · Discussion	Dr. William Stokes, NIH/NIEHS
11:10 AM	***IN VITRO* ENDOCRINE BINDING AND TRANSCRIPTIONAL ACTIVATION ASSAYS: MINIMUM PROCEDURAL STANDARDS AND REFERENCE CHEMICALS** · Public Comments · Committee Discussion	Dr. George Daston, The Procter & Gamble Company
12:05 PM	***LUNCH***	
1:00 PM	**OVERVIEW OF ILSI/HESI SUBCOMMITTEE'S ACTIVITIES ON IDENTIFICATION OF BIOMARKERS OF TOXICITY AND SUMMARY OF FIRST MEETING**	Dr. Dean, Sanofi-Synthelabo, Inc.
1:30 PM	**VALIDATION OF GENETICALLY MODIFIED MOUSE MODELS** · Public Comments · Committee Discussion	Dr. John Bucher, NIH/NIEHS
2:45 PM	***ADJOURN***	

Scientific Advisory Committee on Alternative Toxicological Methods (SACATM)

Daniel Acosta, Jr., Ph.D.
Dean, College of Pharmacy
University of Cincinnati
P.O. Box 670004, 3223 Eden Avenue
136 Health Professional Building
Cincinnati, OH 45267

Rodger D. Curren, Ph.D.
President, Institute for In Vitro Sciences, Inc.
21 Firstfield Road, Suite 220
Gaithersburg, MD 20878

Jack H. Dean, Ph.D.
President and Scientific Director
Sanofi-Synthelabo Research Division
Sanofi-Synthelabo, Inc.
9 Great Valley Parkway
Malvern, PA 19355

Nancy Flournoy, Ph.D.**
Professor, Department of Statistics
University of Missouri-Columbia
222 Mathematical Science Bldg.
Columbia, MO 65211

Alan M. Goldberg, Ph.D.
Director, Center for Alternatives to Animal Testing
Bloomberg School of Public Health
Johns Hopkins University
111 Market Place, Suite 840
Baltimore, MD 21202

Sidney Green Jr., Ph.D.
Graduate Professor
Department of Pharmacology
Howard University College of Medicine
Numa P. Adams Building, Room 3409
520 W. Street, NW
Washington, DC 20059

A. Wallace Hayes, Ph.D.
Science Advisor
Harvard School of Public Health
298 South Main Street
Andover, MA 01810

Nancy A. Monteiro-Riviere, Ph.D.**
Professor, Department of Clinical Sciences
College of Veterinary Medicine
Center for Cutaneous Toxicology
North Carolina State University
4700 Hillsborough Street, Rm D343
Raleigh, NC 27606

Stephen H. Safe, Ph.D.
Distinguished Professor
Department of Veterinary Physiology and Pharmacology
College of Veterinary Medicine, Rm 410
Texas A&M University
College Station, TX 77843

Jacqueline H. Smith, Ph.D.
Chesapeake Consulting Team
25524 Bushey Heath Road
P.O. Box 347
Royal Oak, MD 21662

Carlos Sonnenschein, M.D.
Professor, Department of Anatomy & Cellular Biology
Tufts University School of Medicine
136 Harrison Avenue
Boston, MA 02111

Martin L. Stephens, Ph.D.
Vice President for Animal Research
The Humane Society of the United States
2100 L Street, NW
Washington, DC 20037

Katherine A. Stitzel, D.V.M
8441 Deer Path Drive
West Chester, OH 45069

Peter Theran, V.M.D.
Vice President of Health and Hospitals
Director, Center for Laboratory Animal Welfare
Massachusetts Society for the Prevention of Cruelty to Animals
350 South Huntington Avenue
Boston, MA 02130

Calvin C. Willhite, Ph.D.
Toxicologist
Department of Toxic Substances Control
State of California
700 Heinz Street, Suite 200
Berkeley, CA 94710

**not in attendance

Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

George Cushmac, Ph.D.
Department of Transportation/RSPA
DHM-20
400 7th St., SW, Rm. 8430
Washington, DC 20590

Ms. Patty Decot
Director, Bio-Systems,
Office of Deputy Under Secretary of
Defense for Science & Technology
1777 N. Kent St., Suite 9030
Rosslyn, VA 22209

Kailash Gupta, D.V.M., Ph.D.
USCPSC
4330 East West Hwy, Rm. 600-7
Bethesda, MD 20814

Vera Hudson, M.S.
National Library of Medicine
2 Democracy Plaza
6707 Democracy Blvd.
Bethesda, MD 20892

Mr. Joseph J. Merenda
Director, Office of Science Coordination & Policy
US EPA, EPA East Bldg.
1201 Constitution Ave., NW
Room 4121L
Washington, DC 20004

Alan Poland, M.D.
Chemical & Physical Carcinogenesis
Branch, Division of Cancer Biology
National Cancer Institute
6130 Executive Park Blvd., Suite 5000
MSC: 7368
Bethesda, MD 20892

Leonard M. Schechtman, Ph.D.
ICCVAM, Chair
Assoc. Deputy Director
Washington Operations
DHHS/FDA/NCTR/OD/WO
5600 Fishers Lane, Ste 16-53 HFT-10
Rockville, MD 20857

Margaret D. Snyder, Ph.D.
Office of Science Affairs
National Institutes of Health
9000 Rockville Pike
1 Center Dr., MSC 0162, Rm. 252
Bethesda, MD 20892

William Stokes, D.V.M.
ICCVAM Executive Director
Associate Director for Animal and Alternative Resources
Environmental Toxicology Program
NIEHS
P.O. Box 12233, MD EC 17
Research Triangle Park, NC 27709