MEETING OF THE SCIENTIFIC ADVISORY COMMITTEE ON ALTERNATIVE TOXICOLOGICAL METHODS

JUNE 18 -19, 2008

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I. LOCATION OF BACKGROUND MATERIALS/PRESENTATIONS AND FREQUENTLY USED ABBREVIATIONS

Background materials and presentations for the SACATM meeting are available on the SACATM meeting web site (directly at *http://ntp.niehs.nih.gov/go/7441* or *http://ntp.niehs.nih.gov/* see "Advisory Board & Committees").

3Rs	Replacement, reduction, refinement (causing less pain and distress) in the use of animals for toxicological testing
AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care
ADA	American Drug Association
ADME	absorption, distribution, metabolism, and excretion
ATSDR	Agency for Toxic Substances and Disease Registry
AWIC	Animal Welfare Information Center
BCOP	Bovine Corneal Opacity and Permeability
CEBS	Chemical Evaluation in Biological Systems
CBER	Center for Biologics Evaluation and Research
CPSC	Consumer Product Safety Commission
CRO	contract research organization
DOD	Department of Defense
DOT	Department of Transportation
EC3	estimated concentration needed to produce a SI of three
ECt	estimated concentration needed to produce a SI with a threshold other than 3.0, in order to distinguish between sensitizers and non-sensitizers
ECVAM	European Centre for the Validation of Alternative Methods
ED	endocrine disrupter
EDIT	evaluation-guided development of *in vitro* test batteries
ELISA	enzyme-linked immunosorbent assay
EPA	U.S. Environmental Protection Agency
ESAC	ECVAM Science Advisory Committee
FDA	Food and Drug Administration
FETAX	frog embryo teratogenesis assay Xenopus
FOB	functional observational battery
FYP	NICEATM-ICCVAM Five-Year Plan
FTTW	Future of Toxicity Testing Workgroup
GLP	Good Laboratory Practice
HET-CAM	Hen's Egg Test – Chorioallantoic Membrane
HPV	high production volume
HSLF	Humane Society Legislative Foundation

HSUS	Humane Society of the United States
HTS	high throughput screening
IACUC	Institutional Animal Care and Use Committee
IARC	International Agency for Research on Cancer
ICCR	International Cooperation on Cosmetics Regulations
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ICATM	International Cooperation on Alternative Test Methods
ICE	Isolated Chicken Eye
ILS	Integrated Laboratory Systems, Inc.
IRE	Isolated Rabbit Eye
JaCVAM	Japanese Center for the Validation of Alternative Methods
LLNA	local lymph node assay
MLI	NIH Molecular Libraries Initiative
MOU	Memorandum of Understanding
MTD	maximum tolerated dose
NAS	National Academy of Sciences
NCGC	NIH Chemical Genomics Center
NCI	National Cancer Institute
NCTR	National Center for Toxicological Research
NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NIH	National Institutes of Health
NLM	National Library of Medicine
NRC	National Research Council
OECD	Organisation for Economic Cooperation and Development
OSCP	Office of Science Coordination and Policy
PETA	People for the Ethical Treatment of Animals
R&D	research and development
REACH	Registration, Evaluation, Authorization and Restriction of Chemical Substances
SACATM	Scientific Advisory Committee on Alternative Toxicological Methods
SBIR	Small Business Innovation Research
SD	standard deviation
SI	stimulation index
SOT	Society of Toxicology
STTA	Stably Transfected Transcriptional Activation
TSCA	Toxic Substances Control Act
USACEHR	US Army Center for Environmental Health Research
USDA	U.S. Department of Agriculture
VMO	Veterinary Medical Officer
XDS	Xenobiotic Detection Systems

II. ATTENDANCE

SACATM met on June 18 – 19, 2008, at the Radisson Hotel, 150 Park Drive, Research Triangle Park, NC 27709. The following individuals attended the meeting:

SACATM
James Freeman, Ph.D., ExxonMobil Biomedical Sciences, Inc., *Chair*
Frank Barile, Ph.D., St. John's University
Marilyn Brown, D.V.M., Charles River
Grantley Charles, Ph.D., Allergan
Mary Jane Cunningham, Ph.D., ILS
George DeGeorge, Ph.D., MB Research Laboratories
Helen Diggs, D.V.M., University of California - Berkeley
Michael Dong, Ph.D., California Department of Pesticide Regulation
Donald A. Fox, Ph.D., University of Houston
Daniel Marsman, D.V.M., Ph.D., Procter & Gamble
Roger McClellan, D.V.M., D.A.B.T., D.A.B.V.T., F.A.T.S., Consultant

Liaison Representatives
Jens Linge, Ph.D., ECVAM
Hajime Kojima, Ph.D., JaCVAM
David Blakey, Ph.D., Health Canada

NIEHS/NIH Staff
Eddy Ball
John Bucher, Ph.D., D.A.B.T.
Sally Fields
Dori Germolec, Ph.D.
Debbie McCarley
Sheila Newton, Ph.D.
Barbara Shane, Ph.D., D.A.B.T.
Nigel Walker, Ph.D.
Mary Wolfe, Ph.D.
Lori White, Ph.D. (*Executive Secretary*)

Other Federal Staff
Norka Ruiz Bravo, Ph.D., NIH
Joseph Tomaszewski, Ph.D., NCI

Invited Speakers
Kim Boekelheide, M.D., Ph.D., Brown University
Michael Luster, Ph.D., West Virginia University

ICCVAM Primary Representatives
George Cushmac, Ph.D., DOT
Jodie Kulpa-Eddy, D.V.M., USDA
Karen Hamernik, Ph.D., EPA
Paul Nicolaysen, V.M.D., NIOSH
RADM William Stokes, D.V.M., NIEHS, *Director, NICEATM*
Marilyn Wind, Ph.D., CPSC, *ICCVAM Chair*
COL Peter Schultheiss, D.V.M., DOD
Richard McFarland, M.D., Ph.D., FDA/CBER

Other ICCVAM Representatives
Suzanne McMaster, Ph.D., EPA
Paul Howard, Ph.D., FDA/NCTR
Raymond Tice, Ph.D., NIEHS
Raj Chhabra, Ph.D., D.A.B.T., NIEHS

ILS staff (NICEATM support contractor)
David Allen, Ph.D.
Thomas Burns, M.S.
Patricia Ceger, M.S.
Frank Deal, M.S.
Linda Litchfield
Judy Strickland, Ph.D., D.A.B.T.
Michael Paris
Eleni Salicru, Ph.D.
Cathy Sprankle
Douglas Winters, M.S.

Public
Sara Amundson, HSLF, HSUS
George Clark, XDS
Steven Clayton, Durham
George DeGeorge, Independent Scientist
Adriana Doi, Ph.D., BASF
Dmitry Gazarian, St. John's University
Diane Gerken, Battelle
John Gordon, XDS
John Hamlett, Durham
Sue Leary, Alternatives Research & Development Foundation
Ann-Marie Matel, St. John's University
Kate Willett, PETA

DAY 1 - June 18, 2008

III. WELCOME, INTRODUCTIONS, AND RECOGNITION OF RETIRING MEMBERS

Dr. Freeman, SACATM Chair, called the meeting to order at 8:30 A.M. Individuals in the room introduced themselves. Dr. Bucher, Associate Director of the NTP, made opening remarks. He welcomed everyone on behalf of Dr. Samuel Wilson and introduced Dr. Lori White, NTP Executive Secretary. He said it was a very important meeting as ICCVAM maps out a pathway to move forward from the Five-Year Plan (FYP). He recognized the service of retiring members Drs. Becker, Cunningham, DeGeorge, Dong, and McClellan and the recent passing of Dr. June Bradlaw.

Dr. Wind, ICCVAM chair, welcomed everyone and reviewed ICCVAM's mission. She said ICCVAM facilitates the development of new methods to be used in a regulatory framework. They must do this while maintaining and improving the safety for humans, the environment, and animals. She thanked the members of SACATM for their time and input. Dr. White read the conflict of interest statement for SACATM.

IV. ICCVAM-NICEATM UPDATE

A. Presentation

Dr. Stokes, NICEATM Director and ICCVAM Executive Director, provided an overview of ICCVAM-NICEATM activities since the June 12, 2007 SACATM meeting and highlighted several topics on the current meeting agenda.

10-Year Anniversary Symposium
Dr. Stokes presented highlights of the NICEATM-ICCVAM 10-Year Anniversary Symposium held on February 4, 2008, at the CPSC headquarters in Bethesda. The FYP was released at the symposium, which had over 100 attendees. They held a panel discussion including members from government, industry, academe, animal protection, SACATM, ECVAM, JaCVAM, and the NTP Executive Committee. He reviewed ICCVAM's accomplishments, including the seventeen alternative methods accepted or endorsed by regulatory agencies since 1999. Other accomplishments included developing recommendations for R&D, translation, and validation activities for various alternative test methods; developing international guidance on test method validation and acceptance criteria and processes; defining and establishing a process for development of performance standards; and establishing and strengthening international partnerships with ECVAM and JaCVAM.

NICEATM-ICCVAM Five-Year Plan
The NICEATM-ICCVAM FYP was released at the 10-Year meeting and was forwarded to Congress on February 4, 2008. It emphasizes priority areas for the 3Rs, application of new science and technology, partnerships, and international cooperation and harmonization.

International Cooperation on Alternative Test Methods (ICATM)
Dr. Stokes explained the proposal for a new ICATM, the purpose of which is to promote *consistent* international cooperation, collaboration, and communication among national validation organizations (ICCVAM-NICEATM, ECVAM, JaCVAM, Health Canada). ICATM's goals are to ensure optimal design and conduct of validation studies, ensure high quality independent scientific peer reviews, enhance likelihood of harmonized recommendations by national validation organizations, and leverage limited resources to achieve greater efficiency and effectiveness and avoid duplication of effort.

Alternative Methods for Ocular Safety Assessments
Dr. Stokes discussed the *ICCVAM Test Method Evaluation Report: In Vitro Ocular Toxicity Test Methods for Identifying Severe Irritants and Corrosives*. ICCVAM recommended four ocular alternatives to agencies and all were accepted or endorsed by applicable agencies. These are the first non-animal alternative test methods for ocular safety testing to be accepted by regulatory authorities. Planned activities include improving the Bovine Corneal Opacity and Permeability (BCOP) and Isolated Chicken Eye (ICE) test methods to increase their accuracy, evaluating *in vitro* methods proposed to identify substances that cause reversible eye damage, assessing integrated decision strategies using data from multiple methods and biological activity information, evaluating non-animal approaches for determining ocular hazard potential of antimicrobial cleaning products, and reviewing the use of topical anesthetics and systemic analgesics.

Alternative Methods for Acute Systemic Toxicity
Dr. Stokes provided information on the International Workshop on Acute Chemical Safety Testing, held on February 6 and 7, 2008 at the Natcher Conference Center, NIH, Bethesda, MD. The workshop, organized and sponsored by NICEATM, ICCVAM, ECVAM, and JaCVAM, had over 120 participants and a goal of determining how key *in vivo* toxicity pathway information can be collected and used to develop more predictive mechanism-based *in vitro* tests and earlier, more humane endpoints.

Also in February, ICCVAM released its Test Method Evaluation Report on *In Vitro Cytotoxicity Test Methods for Estimating Starting Doses for Acute Oral Systemic Toxicity Tests.* ICCVAM recommends that the *in vitro* tests should always be considered before using animals for acute oral toxicity testing, and that the tests should be used where determined appropriate. Current and planned activities are development and evaluation of an Up-and-Down Procedure for acute dermal systemic toxicity, assessment of reduction methods for acute inhalation toxicity, evaluation of the 3T3 NRU cytotoxicity method for estimating starting doses for the acute oral toxicity testing of mixtures, and further evaluation of the usefulness of an *in vitro* limit dose to identify nontoxic substances.

Alternative Methods for Allergic Contact Dermatitis
A peer review meeting was held at CPSC headquarters on March 4 - 6, 2008, to evaluate modifications and new applications of the local lymph node assay (LLNA). Current and planned activities are finalizing the limit dose procedure BRD and ICCVAM test method evaluation report, working to harmonize LLNA performance standards with ECVAM and publishing the final recommendations, requesting additional existing data for the non-radioactive modified

LLNA methods and updating those BRDs, reconvening the peer panel, and updating the OECD Test Guideline 429.

Alternative Methods for Biologics Testing
Dr. Stokes reported on the Scientific Workshop on Alternative Methods to Refine, Reduce, and Replace the Mouse LD_{50} Assay for Botulinum Toxin Testing that was co-sponsored by ICCVAM, NICEATM, and ECVAM. A report of the workshop with summaries of the presentations and panel discussions was prepared and is available on the ICCVAM website. ICCVAM is also planning to evaluate alternatives for vaccine potency testing and is currently awaiting completion of USDA studies on an alternative method for potency testing of veterinary vaccines for *Leptospirosis.*

Alternative Testing Methods for Endocrine Disruptors
The International Validation Study of the LUMI-CELL® Estrogen Receptor Transcriptional Activation Assay was organized by NICEATM, ECVAM, and JaCVAM and is being conducted with 78 chemicals. There will be an independent peer review and submission of an OECD Test Guideline proposal in 2009.

Alternative Methods for Genetic Toxicity Testing
Dr. Stokes discussed the Draft OECD Test Guidelines 487 for the *in vitro* micronucleus assay and stated that several members of the ICCVAM Genetic Toxicity Working Group participated in the October 2007 OECD expert consultation meeting. They continue to contribute efforts to harmonize the approach for evaluating cytotoxicity. The working group has also provided comments on the JaCVAM *In Vivo* Comet Assay Validation Study.

Alternative Methods for Dermal Safety Assessments
Studies are planned to determine how *in vitro* dermal irritation test methods (i.e., EpiDerm™ and EPISKIN™) will classify false negative chemicals from *in vitro* corrosivity test methods. ICCVAM will be evaluating a draft OECD Test Guideline for an *in vitro* skin irritation assay in the next few months. ICCVAM and NICEATM have also submitted proposals to OECD to update their test guidelines for *in vitro* skin corrosion to include performance standards.

6th World Congress on Alternatives & Animal Use in the Life Sciences
Outreach activities included participation in the 6th World Congress on Alternatives & Animal Use in the Life Sciences that was held in Tokyo, Japan on August 21 -25, 2007. Twelve participants representing seven ICCVAM agencies and NICEATM provided 23 presentations and chaired 7 sessions. The 7th World Congress is scheduled for August 30 – September 4, 2009 in Rome.

2*008 Society of Toxicology (SOT) Meeting*
NICEATM-ICCVAM had five poster presentations titled: NICEATM-ICCVAM Five-Year Plan, Performance Characteristics of the LLNA Limit Dose Procedure, ICCVAM Recommendations on *In Vitro* Pyrogen Test Methods, *In Vitro* Methods for Estimating Rat Acute Oral Toxicity: Prediction of GHS Categories, and Alternative Use of *In Vitro* Test Data to Determine When Rat Acute Oral Toxicity Should Start with the Limit Test. For next year's meeting they have submitted proposal for two workshops on alternative methods. Dr. Stokes considers SOT to be

an excellent forum for ICCVAM and NICEATM to provide information to the toxicology community on scientifically valid new, revised, and alternative test methods that have been accepted by regulatory authorities.

B. SACATM Discussion

Dr. Freeman asked how successful the effort has been to improve the consistency of the cooperation on approaches to developing alternative methods. Dr. Stokes said when ICCR gave them the charge; this provided the stimulus to come together to address how the validation organizations, including the representative from Health Canada, could work together more efficiently and consistently. Dr. Stokes said working together the validation organizations have been enormously successful. For example, the joint NICEATM-ECVAM validation study on the two cytotoxicity methods went very well and they developed standardized test method protocols, They also agreed on the chemicals that were used in the validation study design, which led to agreement on the outcome of these validation studies with regard to usefulness and limitations. Dr. Stokes said working together from the start on something makes it easier to come to agreement on the utility of the method and what its limitations are based on the results of the validation study. Agencies need that information to make statements about using these methods to meet their requirements.

Dr. McClellan stated that this is a broad issue, one that will be touched on in the individual agency presentations. The question he requested to be approached broadly was that much of the focus in terms of test methodology is addressed to the question of safe vs. not safe, a "yes/no" issue. The issue is not safe or unsafe, but what is the potency of the material. He requested comments on the extent to which, as the program moves forward, they are going to be able to address some additional attention to that very critical issue. He said he will comment later that it was a serious deficiency in the NRC's recent report. Dr. McClellan said in looking to the future, it is going to be important to consider potency of some materials and to no longer use the artificial distinction between safe and unsafe. Dr. Stokes agreed that it would be a challenge. The first test they looked at asked whether a substance causes corrosion or not, a yes/no answer, but as they have moved toward systemic toxicity, the safety assessment became more quantitative. For example, for acute oral toxicity, there are six different hazard categories based on potency, each driving a different level of hazard labeling and risk management practices such as child-resistant packaging and safety packaging for transportation purposes. Dr. Stokes said they deal with it to some extent on the local toxicities. While only one dose is tested, there are different levels of severity that are used for determining hazard classification. In the high throughput screening (HTS) testing system, which Dr. Tice is overseeing as acting head of the Biomolecular Screening Branch, they use 14 different concentrations in those cell systems. There is quite a significant dose response, even in that approach.

Dr. DeGeorge asked Dr. Stokes to estimate when ESAC, ECVAM, and JaCVAM would mutually recognize and accept each other's validation studies. Dr. Stokes responded that there have been suggestions for many years that there should be reciprocity between organizations so that each would automatically accept the conclusions of the other. However, Dr. Stokes said each organization has completely different processes and regulations for reaching conclusions on scientific validity for regulatory purposes that do not allow for automatic acceptance. In this

country, when reviewing the validity of a new method, it is a very open transparent process; all the materials are made available in the public domain for people to look at and comment on. ICCVAM has an open public peer review meeting, and while it takes longer, in the end, everyone has had the opportunity to comment on the science of that method. However, Japan and Europe do not have similar transparency in their processes, and currently do not hold public meetings or solicit public comments during their review processes. Nonetheless, all of the validation organizations are trying to come up with a way to ensure that when one conducts a peer review, there is opportunity for those materials to be widely available for stakeholder comments at the same time that they are provided to the people on the peer review panel. If those opportunities were provided, then the other organizations might not have to repeat a completely separate peer review panel. Automatic adoption is not something that any organization can do, but if all of the organizations work together, there is a very good likelihood of coming up with similar recommendations. He said that is what ICATM is designed to do, to work together, to have joint discussions, share information, and hopefully at the end of the process have harmonized recommendations that are produced by each organization. Dr. Stokes said ESAC recently agreed to make background review documents available publicly at the same time they send them to their peer review panel. This will allow the opportunity for other stakeholders to make comments and provide them to the peer review panel for their consideration before the review panel finalizes their recommendations. He said this recent change would be very important to progress towards harmonized recommendations.

V. REGULATORY ACCEPTANCE AND AVAILABILITY OF ICCVAM-RECOMMENDED ALTERNATIVE TEST METHODS

A. Presentation

Dr. Stokes reported on the availability and regulatory acceptance of ICCVAM-recommended alternative test methods. The ICCVAM recommendations on *In Vitro* Ocular Toxicity Test Methods for Identifying Severe Irritants and Corrosives were transmitted to 15 agencies on October 26, 2007. All agencies concurred with the ICCVAM recommendations. These are the first validated *in vitro* alternative test methods for ocular safety testing accepted for regulatory use. Dr. Stokes explained that ICCVAM recommends, in accordance with USDA Animal Welfare Act regulations, that these methods should always be considered *before* using rabbits for ocular safety testing and that the alternative methods should be used where determined appropriate. The BCOP and ICE are recommended for use in a tiered-testing strategy, where positive substances can be classified as ocular corrosives without the need for animal testing. The Isolated Rabbit Eye (IRE) and Hen's Egg Test – Chorioallantoic Membrane (HET-CAM) are not considered to *currently* have sufficient performance and/or sufficient data to substantiate their use for regulatory hazard classification purposes, but may be useful for other purposes. ICCVAM encourages industry to use BCOP and ICE, to submit the *in vitro* and any *in vivo* data to NICEATM, and to forward histopathology results or to submit tissues for histopathology examination. ICCVAM and NICEATM are developing draft test guidelines for submission to OECD in July 2008 and consideration is expected at the National Coordinator Meeting, March 31-April 2, 2009. If adopted by OECD, these will be the first non-animal test methods for ocular toxicity accepted for international use.

Dr. Stokes presented the background on two *in vitro* methods that can be used to estimate starting doses for acute systemic toxicity studies. Recommendations were transmitted to agencies on February 28, 2008. Two agencies have responded, both concurring. Use of these methods does not require regulatory acceptance because data will not be used for regulatory decisions. ICCVAM recommends that (1) the assays should be used in a weight-of-evidence approach to determine starting doses for current acute oral toxicity protocols; (2) the methods should be considered before using animals for acute oral toxicity studies and should be used where determined appropriate; (3) the methods can result in reduction and refinement; (4) the methods are not sufficiently accurate to predict acute oral toxicity for the purpose of regulatory hazard classification categories; (5) the methods will likely overestimate starting doses for substances with certain toxic mechanisms, and therefore it may not be appropriate to use the methods for such substances; (6) additional comparative *in vitro* basal cytotoxicity data should be collected; and (7) all *in vitro* and *in vivo* data should be submitted to NICEATM to expand the validation database for these methods and to assist in determining additional assays that will be needed to accurately predict acute oral toxicity hazard without animals.

Dr. Stokes asked SACATM for advice on increasing awareness of these methods, encouraging their use, and encouraging data submission and optional activities.

B. Public Comments

Sara Amundson, HSLF and HSUS, said the difficulty in registering for public comments is that she did not know what was going to be discussed. She asked if there had been thought given to outside, e.g., through Animal Welfare Information Center (AWIC) and literature searches, to have NIEHS proactively reach out to USDA, in conjunction with their own VMOs, and for attending veterinarians and chairs of IACUCs to run a series of workshops on what the existing methods are to ensure that there is something more than a literature search that makes this whole conceptualization realistic to these individuals. She said it is not always about regulatory acceptance because it may not be used for that purpose like the acute toxicology methods. At an animal welfare meeting with Dr. Kulpa-Eddy, she thought it a good opportunity for a collaborative federal government effort to perform outreach. She mentioned that there are two states now that require the use of ICCVAM-approved methods. Other outreach activities with SOT and otherwise, and conducting proactive hands-on workshops would be important. She said there is a grave underutilization of literature searches to meet the requirements under the Animal Welfare Act. She appreciated that ICCVAM always includes the parameters of the Animal Welfare Act, including the regulations and requirements under PHS Policy and AAALAC accreditation that consistently address all species of animals.

Dr. Stokes responded that the idea of having workshops is very good. Previously when ICCVAM has made recommendations on methods, they also held implementation workshops to bring together regulators to look at the data from the methods as well as the scientists and toxicologists who will be carrying out the methods or asking contract laboratories to conduct them. This is valuable to help them understand how to conduct tests, how to interpret results, what the limitations are, and how it can currently be used. He said he appreciated the suggestion and that workshops are proposed for SOT in 2009. ICCVAM representatives have engaged the leadership of SOT in discussions on the importance of such workshops at SOT so that they

would be more receptive to accepting these types of workshops. He said the suggestion for providing training to the technicians performing the assays is also important. He invited Dr. Kulpa-Eddy from USDA to provide information about the training of VMOs who do inspections and review IACUC records to ensure compliance with the Animal Welfare Act.

Dr. Kulpa-Eddy said Dr. Stokes had spoken at VMO research training courses and it had been very informative for them understand what's been approved through ICCVAM and what they can be looking for when they do their inspections at the research facilities, academic facilities, pharmaceutical firms, and CROs. They have, in response to the ICCVAM recommendations, put this information on their AWIC website, which is their outreach to the research community. They are open to other suggestions. Dr. Brown asked for clarification of the public comment regarding underutilization of literature searches. Dr. Kulpa-Eddy responded that the consideration of alternative methods citation is the one of the most common citations that they see, about 7% of the inspection reports. She added that they have about 1,100 registered research facilities in the United States and they conducted about 1,600 inspections last year. Of those, 70% had no non-compliant items listed on their inspection report. The remaining 30% commonly were lacking consideration of alternative methods.

Dr. Brown asked Dr. Stokes about impediments to the collection of parallel data. She mentioned doing a GLP study for submissions and the sponsor not wanting any information collected that does not go into the FDA submission. She asked if they were being put between a rock and a hard place in terms of wanting them to collect this information, but then having concerns about that information going to the FDA and being problematic in any way. Dr. Stokes responded that ICCVAM is proposing that labs collect the histopathology data, not make decisions about it or interpret results, but simply collect and process it. So it would not be part of the GLP submission, it would be ancillary information. Dr. Brown said the sponsor would own any data collected, so it would be the sponsor's decision about sending the data to ICCVAM. Dr. Stokes said the sponsors would have to authorize collection of that ancillary data and authorize it to be forwarded to ICCVAM. Dr. Brown said sponsors would be a target audience to reach out to for this information.

C. SACATM Discussion

Dr. White read Dr. Becker's written comments regarding increasing awareness of alternative methods: "There are a number of approaches that may prove to be useful that could be considered, such as: development of symposia or a continuing education course as part of the annual meeting of professional societies (such as SOT, American College of Toxicology or American Association for Laboratory Animal Science); collaborate with IACUC (*www.iacuc.org)*; development of web-based education and outreach materials to include identification of the specific regulatory applications where such methods have been deemed acceptable by the Agencies; consider having each ICCVAM agency have a web page on their web site on "Agency Activities to Reduce, Refine or Replace Laboratory Animals in Testing and Research" and focused outreach to sectors where such testing is most often employed through their newsletters etc. (professional societies (SOT, ACT, ISRTP, etc.) and trade groups (American Chemistry Council, Personal Care Products Council, Soap and Detergent Association, Consumer Specialty Products Assoc., CropLife America, etc.)." Regarding question 2, Dr.

Becker referred to his previous response about education and outreach and referred to the discussion of Breakout Group 5 of the Acute Safety Testing Workshop.

Dr. Diggs, a lead discussant, said her comments were redundant to Dr. Becker's. She suggested an educational guidance document that could be distributed to different associations and organizations. It would summarize the changes, give a history of the ICCVAM program, review the efforts in a summarized way, make it very clear where they are headed, and outline the expectations and standards for the program directors, IACUC coordinators, and institutional officials. Another venue might be the training programs that are already there for IACUC. Getting it to the hands of the IACUC chairs and coordinators is crucial, because they are the ones doing protocols and ensuring appropriate review by the committee. Other organization might be the American College of Laboratory Animal Medicine (ACLAM), and the American Society for Laboratory Animal Practitioners, AAALAC, and AWIC. Dr. Diggs asked Dr. Stokes about the histopathology data that Dr. Brown mentioned in terms of the financial impact on the program directors. Dr. Stokes answered that the time it takes to collect and process the tissue does incur cost. It is something that is completely voluntary. ICCVAM is encouraging it because they feel it is a valuable way to help further characterize the usefulness of the methods and also help generate the database that is needed to determine how one can use the histopathology to increase the accuracy of predictions. If organizations want to contribute to the effort, this is one way they can do that. It does involve extra staff time, which would incur a financial cost. Dr. Diggs asked about concerns regarding forwarding proprietary information. Dr. Stokes agreed regarding concerns about testing being proprietary and said ICCVAM would like to know the product or substance that has been tested, but they don't want to be receiving confidential business information, so they have asked sponsors to provide whatever information they can about the substance. Sometimes it is limited to physicochemical properties or a code name for a chemical.

Dr. Barile said he was concerned with the response letters from FDA and EPA. He cited the FDA letter, "FDA does not prescribe specific test methods for cosmetics. Rather sponsors of cosmetic products have a general requirement to determine safety by those methods that they deem appropriate." He said ICCVAM cannot mandate these requirements, but an agency that is responsible for product testing and drug development and for overseeing public health and drugs that enter the marketplace has made a rather weak response to methods that have been evaluated. He said the EPA asked ICCVAM to evaluate the ocular testing methods. He cited the EPA letter is a much more forceful reaction and implies that they will take the opportunity to present these methods to companies that are required to do product testing. He said he thinks this is a missing link in SACATM's and ICCVAM's work, to make the public and regulatory agencies more aware and encourage the use of recommended methods.

Dr. McFarland responded that each letter is a reflection of the differences in the way the underlying regulations are written between the two agencies. He said he would not comment on the EPA response because he is not familiar with EPA regulations. However, 10 years ago, FDA did a fairly extensive rewriting of regulations to remove, in many cases, prescriptive language requiring specific tests. FDA believes it is more effective to require companies to have regulations that prescribe that they meet general requirements for determining safety, rather than prescriptive methods, which then allows FDA to accept data from newly derived methods, methods that keep up with the technology. The key is within the agency communicating the availability of methods.

Dr. Brown suggested that ICCVAM could develop a short handout or a leaflet that would be available on the web and could be given out at OLA, IACUC 101, or at meetings where AWIC is exhibiting. It would be very useful to have a short guidance document that could describe the kind of data being requested. She said speaking from a CRO environment, it would be something to take to a sponsor and say, "This is what we're trying to do, this is what it involves." Regarding the letter from the FDA and the question about how strong the regulatory agencies seem to be pushing or encouraging alternatives, just as for IACUC proposals, they have to say why alternatives are not used or not appropriate, and how the decision was made. Perhaps in submissions to regulatory agencies, there could be a similar kind of question. The regulatory agencies would ask, "Were alternatives available? How did you determine they could not be used in this particular case?" If they have to scientifically justify the use or non-use of these alternatives, that would really go a long way to increasing people's awareness and getting their attention.

Dr. Cunningham echoed Drs. Brown and Dr. McFarland's comments on encouraging optional data. The regulatory agencies vary in how they look at new alternative data on new methods. She suggested looking at how the FDA recently put together a formal process for looking at pharmacogenomics and toxicogenomics data. Even though those data have to be part of the submission for the applications, they've created a formal process to look at those optional data. She suggested looking at how that process was set up and what they are doing with the data.

Dr. DeGeorge noted that the strength of responses from agencies differed markedly. He thought the agencies should provide stronger responses. He then discussed the recommendations that histopathology be included in ocular studies, acknowledging that it would increase costs, and he added that until a requirement for histopathology is put into agency guidelines, companies would not do it.

Dr. Freeman asked if any thought had been given to having tissues sent to NICEATM and having NICEATM sponsor the pathology. Dr. Stokes responded that the NTP does have considerable expertise in pathology and quite a few veterinary pathologists on staff and available via support contracts. He said it is something they could consider. He appreciated the comments about the different levels of expense needed, e.g., collecting and preserving tissues versus processing and interpreting the results. As you progress down the hierarchy of activities your costs go up. He said that may be one approach to making it a reasonable activity and thanked SACATM for the suggestion.

Dr. Fox said a shortcoming is that there are no guidelines or criteria as to how the histopathology should be done. There are a variety of methods for fixation and staining that can provide differing results. When the data came to NICEATM, they would have some organization and some standard protocol to look at to see how this worked and compare it to standards developed with collaborators or outside sources. He said NICEATM might have to do testing themselves. Dr. Stokes said the panel recommended that ICCVAM develop guidance on histopathology, including how to collect, preserve, and process tissues. NICEATM has begun working with the NTP pathologists to develop this guidance, which will be in a document that will accompany the OECD test guidelines.

Dr. McClellan suggested trying to get something out in the open peer-reviewed literature, such as an op-ed piece for *Toxicological Sciences* and a broader review article for *Critical Reviews in Toxicology*. He expressed concern regarding the issue of voluntary or optional collection and reporting of data. He suggested backing up and asking what would be done with those data? They will be nonrandom and almost of no use from a scientific standpoint. He mentioned how agencies differ in terms of their regulatory requirements. ICCVAM is really a cooperative venture, there is very little that requires these agencies to do anything as they participate in ICCVAM, except make a good faith effort. He mentioned that in 2001 EPA announced the availability of the *in vitro* methods and encouraged their use in the high production volume (HPV) program. He asked if anyone in EPA could tell him what the response has been. He said it links to Dr. Stokes' slide 13, stating that there was encouragement in terms of *in vitro* and *in vivo* data being submitted to NICEATM for further analysis. He asked what EPA found in response to that encouragement for the HPV program and what is the linkage between that kind of action by a specific agency and the interagency kind of approach.

Dr. Stokes said when EPA sent the letter to the companies that would be generating data under the HPV initiative; they encouraged them to submit the data to NICEATM. The summary data were to be made available to the public. NICEATM did not receive any *in vitro* data; only one data set was made available in summary on the HPV website. He said there was not a large response and they should look at why they didn't get a response; however, he didn't think there was a lot of acute oral toxicity testing conducted under the HPV. Dr. McClellan expressed concern about a "cherry-picking" of the results and a reluctance of organizations to submit data. He stressed that the data must be scientifically valid. Dr. Stokes said he appreciated the comments and that they do have to be cautious in understanding the biases and the type of data received under that voluntary program. One of the precepts that ICCVAM follows is to consider all of the available data that exist on a test method when reviewing its usefulness and limitations. They sometimes get data only on certain product or chemical sectors and none in other areas.

Dr. Hamernik said that the HPV program is largely a voluntary program. She said one of the difficulties in the Toxic Substances Control Act (TSCA) is getting data, even for EPA purposes, and in some cases involves lengthy rule writing. Dr. Wind said breakout group 5 at the recent ICCVAM workshop on acute toxicity dealt with the issue of how to get data from industry and why the response to the EPA request was not met. The basic answer was that industry already had the animal data, so there was no point in doing *in vitro* tests for chemicals for which they knew the answer. She said there was an interest in working with industry and seeing what they could do to facilitate such a transfer of data. Dr. Hamernik said she is co-chair of the ICCVAM Ocular Toxicity Working Group. She said the working group took the expert peer review panel's recommendations very seriously with regard to developing standardized procedures for histopathology, for *in vitro* ocular methods, and for developing an atlas or procedure for doing pathology, and they were enthusiastic about following up on those recommendations.

Dr. Charles stated that as a sponsor he would like to give industry's perspective in response to Dr. Barile's and Dr. McFarland's comments. He provided an example of the importance of encouragement by the regulatory authorities. His colleague recently submitted a test plan. The agency commented on the proposal to use the guinea pig maximization test and suggested he consider the LLNA instead, which resulted in doing six LLNAs instead of six guinea pig

maximization tests. He said input from the agency is important; just a recommendation or suggestion might be enough for a sponsor to go forward with an alternative test. If the agencies basically go forward with the suggestions and filter this information down to the people looking at the plans and reviews, it's a significant help. He said, in terms of using the *in vitro* basal cytotoxicity evaluations for setting starting dose levels, from a sponsor's perspective, these studies are typically done at CROs. If the CRO has a system in place for setting the doses on the studies, the sponsor probably will not argue that much in terms of utilizing it. In many cases the sponsor will use the CRO as its adviser for the toxicological testing. To encourage use of the *in vitro* basal assay, target the CROs and the large contract labs. To promote the use of ocular irritation assays, two other venues would be the Society of Toxicological Pathologists and the visual sciences societies like the Association for Research in Vision and Ophthalmology.

Dr. Marsman said he wanted to expand on Dr. Brown's great recommendation to turn the tables and ask investigators why they are not using an alternative method that ICCVAM recommended. In response to the histopathology discussion, he said his company would love to do more histopathology, but expressed concern about the number of chemicals. Further, the ability of ICCVAM to identify the gaps in the data sets so that the registrants specifically know where data are truly needed is the antithesis of what Dr. McClellan said. It is nonrandom, but it is directed at filling a particular void so there is more buy-in. He disagreed with Dr. McClellan and thought it valuable that ICCVAM can identify the gaps in the data sets so that registrants specifically know where data are needed.

Dr. Brown asked about the connection between the recommendations for dose-setting and using *in vitro* methods for dose-setting in acute systemic oral toxicity studies and how it relates to the article from the United Kingdom and the center for the 3Rs that questions the usefulness of the acute oral toxicity test. Dr. Stokes said the decision not to require or use estimates of LD_{50} for hazard classification categories is a regulatory decision, not an ICCVAM decision. ICCVAM makes recommendations on methods that can be used to meet current regulatory requirements. If regulatory authorities move away from estimates of LD_{50}, then they are going to use something that indicates some level of toxicity. Whatever that target is, that is what these *in vitro* methods should be able to predict. In many cases what companies and regulatory authorities want to know is what dose level causes some kind of adverse effect, not necessarily death. He said he thinks that's the gist of the U.K. report.

Dr. Brown suggested that an expert working group critically review the U.K. report. Dr. Stokes clarified that Dr. Brown said the report suggests that 14- or 30-day subchronic studies with a lower dose may provide information about systemic toxicity that would negate the need to do an acute oral single high dose study.

VI. OVERVIEW OF NICEATM-ICCVAM FIVE-YEAR PLAN

A. Presentation

Dr. Wind presented an overview of the NICEATM-ICCVAM FYP. Specifically, the plan resulted from a request from the U.S. Senate and House of Representatives Appropriations Committees that NICEATM and ICCVAM develop a five-year plan in conjunction with federal

agency program offices to foster and promote research, development, translation, and validation of alternative test methods that will reduce, refine, and replace the use of animals for safety testing, while maintaining scientific quality and the protection of human health, animal health, and the environment. Preparation of the FYP was a three-phase process that started in August 2006 and was completed in February 2008. A great deal of public and SACATM comment was incorporated into the plan.

The FYP is "a plan to advance alternative test methods of high scientific quality to protect and advance the health of people, animals, and the environment." It builds on the NTP Roadmap, to "develop and validate improved testing methods and, where feasible, ensure that they reduce, refine, or replace the use of animals," and is consistent with the recent National Academy of Sciences Report: *Toxicity Testing in the 21st Century: A Vision and Strategy*. The FYP further builds on current U.S. laws and policies regarding the mandate to protect human and animal health and the environment; to determine if alternative methods can provide equal or better protection before their adoption or endorsement; and to reduce, refine, and replace existing animal testing with alternatives where appropriate.

She explained the role of NICEATM and ICCVAM to promote and facilitate research, development, translation, and validation activities with the 15-member federal agencies. She emphasized that ICCVAM and NICEATM do not have laboratories or resources to carry out research and development activities, but depend on federal research laboratories and other stakeholder organizations to develop new test methods. These are the four key challenges:

(1) Identifying priorities and conducting and facilitating activities in priority areas, which currently include ocular, dermal, and acute toxicity; biologics and vaccines; immunotoxicity; endocrine disruption; pyrogen testing; reproductive/developmental toxicity; and chronic toxicity/carcinogenicity testing. She explained the bases for priorities and planned activities.
(2) Identifying and promoting new science and technology in areas including HTS, other animal systems, computational approaches, biomarkers of toxicity, nanomaterials testing, and toxicology databases. Eleven agencies have R&D programs that ICCVAM will monitor.
(3) Fostering regulatory acceptance and use of alternative test methods by providing guidance and comprehensive test method evaluations, carrying out independent peer reviews, and organizing implementation workshops.
(4) Developing partnerships with stakeholders to leverage resources; maximize efficiency/minimize duplication of efforts; ensure early exchange of information; facilitate national and international recognition, acceptance, and implementation of scientifically valid test methods; and collaborate with ECVAM and JaCVAM to carry out independent validation studies and test method evaluations.

Initial implementation activities included an acute chemical toxicity workshop, a proposal for International Cooperation on Alternative Testing Methods (ICATM), the ICCVAM Research and Development Working Group and the ICCVAM FYP Implementation Subcommittee. Dr. Wind articulated the FYP goals: (1) further reduction and replacement of animal use where scientifically feasible, (2) further reduction or elimination of pain and distress where animals are still used, and (3) continued and improved protection of public health, animal health, and the environment. She said ICCVAM looks forward to SACATM's advice on priorities and activities as they continue implementation of the plan.

B. SACATM Discussion

Dr. Charles, a lead discussant, congratulated ICCVAM on the ambitious FYP. He recommended that ICCVAM focus on activities where they can have the highest impact and set metrics that they think they can achieve with defined milestones. Later, they can identify the metrics and milestones that have been met, so in five years they can easily tabulate what was accomplished. He also commended ICCVAM on the approach to harmonization. Working internationally to get harmonized guidelines for test systems across international boundaries will be a very good aid to getting implementation by industry, instead of industry having to do multiple tests for different countries or regions. He was glad that ICCVAM would be involved in the OECD validation process, working on OECD-based guidelines, protocols, and the test methodologies that are performed to a large degree by industry. In terms of fostering acceptance, from an industry perspective, he mentioned that CROs are doing more and more of the testing and some are involved in test method validations. He gave as examples the comet assay and the *in vivo* micronucleus test system. He said emphasis has to be placed on interactions with the CROs, getting them onboard, because many of them act as the experts in their fields for industry. He said the toxicology database is a good idea and wondered if a Genbank-type concept of data submission was ever considered, e.g., peer-reviewed data that the authors could submit by filling out a form so they don't have to use a separate process. ICCVAM would not have to call separately for data and it would facilitate updating the database more rapidly.

Dr. Marsman, a lead discussant, said he was pleased with the revisions to the FYP and the priorities are reasonable, based upon animal welfare and investigator needs. One area where there could be greater emphasis is for ICCVAM to encourage not just replacement methodologies, but refinement and reduction methodologies. He said it is not clear in the FYP that the other two Rs are important. He highlighted under refinements the new methodologies, such as subclinical endpoints, that may allow an investigator to terminate the study sooner rather than later. Under reduction, he mentioned new technologies, such as microarrays, that could complement *in vivo* studies. He asked ICCVAM to emphasize all 3Rs. Under research priorities, he found the FYP lacking detail. Moving into challenges three and four, he found overlap. He said the plan falls short due to the lack of specifics and suggested identifying the stakeholder needs based on what the regulatory agencies around the world are requiring. He said U.S. agencies' expectations are different, and the greater clarity that ICCVAM can bring to identifying what those expectations are would help set the game for strategies for alternative methods. If that vision is not in mind from the start, the assays will always miss their mark, which triggers us back to the default, having to run the *in vivo* test to satisfy one agency or in particular one country. He suggested identifying the stakeholder needs for the registrant and all the varying expectations of the different agencies and countries.

Dr. Brown, a lead discussant, agreed about refinement not being included in the challenge. She agreed with Dr. Marsman that there is a lot of room for improvement in the near term. She saw a need for a mechanism to validate earlier endpoints to refine the studies being done today. She said they had already talked about partnerships with the stakeholders, not so much international stakeholders, but the stakeholders in the United States, pharmaceutical companies, chemical companies, CROs, and getting the message to them. She mentioned the discussion earlier about

providing more information about guidelines or expectations regarding submission of a study or a methodology for validation, as well as the kind of information needed and how to get it, and then sharing the information that is already available including what is in the pipeline, with IACUCs and the scientific community. She said those were the key points she saw, and the FYP does not have a lot of details. It is a broad plan, and the devil is in the details. Dr. Stokes said NICEATM and ICCVAM were directed to limit the length of the report, which did not allow for more detailed information and plans to be included. He said the implementation of the plan is now being initiated.

Dr. McClellan extended his compliments to everyone involved in the process and said he views the plan as primarily aspirational. He said it is not an implementation plan. Most frequently the process is more important than the product; i.e., what did the participants gain from their involvement in it? He said ICCVAM had the challenge of two issues. One is how to deal with Congress regarding the length of the report or the mention of dollars or resources. A plan without resources associated with it is almost worthless; resources must be brought into the question. He said the question is, does ICCVAM have the resources to achieve its aspirations. He said interagency activities are a challenge because each agency has its own mandates and own turf, and in the case of ICCVAM, there are 15 agencies with different statutes. He said the implementation plan should be documented and given as much scrutiny as the FYP. It will be critical that it includes quantifiable, measurable goals in terms of what is to be achieved and when it is to be achieved, not the outcome, but the action on it. He said he thinks it is important to understand that our most important concern is the safety of people and the environment. This activity is clearly concerned with the 3Rs, but we need to recognize where we are headed. He said he is concerned that throughout many of these activities, in the issue of validation, we have fallen into a trap of tying results to the two-year chronic bioassay used by NTP. He said what is really needed is validation relative to known human toxicants, or in that case, carcinogens. He said he repeatedly sees reference to validation in terms of comparison with existing methods, and then, "Oh, by the way, we want to make certain we are protecting human health." He said he fails to see in the FYP, which he expects at the aspirational level, the clear linkage to known human toxicants in terms of validating the methodology. Dr. McClellan mentioned the distinction between hazard identification and the much more robust data set required in terms of dose-response. To date, ICCVAM activities have focused on eye and skin, areas where the distinction between exposure and dose are not nearly as complex as with inhaled or ingested agents. He suggested this important issue be given attention in the implementation plan. He acknowledged that on some occasions he has been critical of the progress ICCVAM made, but now recognizes that it was probably resource-constrained. He said ICCVAM needs to actually document what the resources have been, which will give a better basis for anticipating what can be done in the future. He looked forward to seeing the implementation plan and urged that it be given the same kind of scrutiny and public and committee review as the FYP was given.

Dr. Freeman said these comments reflected some of the initial comments from a year ago, a lot of which were addressed. He asked about the status of the implementation plan and when SACATM would have the opportunity to see it. Dr. Stokes answered that some of the initial implementation activities that have been undertaken were mentioned. The first occurred two days after the plan became available: the Workshop on Acute Chemical Safety Testing: Advancing *In Vitro* Approaches and Humane Endpoints for Systemic Toxicity Evaluations. The

workshop focused on how to collect more mechanistic *in vivo* information to inform the development of predictive *in vitro* systems, and to help identify earlier, more humane endpoints. The development and use of early more humane endpoints is an important way to lessen pain and distress, which is refinement. He said ICCVAM recognizes that refinement can be attained much more quickly than replacement, and in some cases, even enable further reduction in animal use. ICCVAM will definitely work on all three of the Rs. He addressed the implementation plan, stating that the ICCVAM working groups in each of the topic areas would be asked to work with NICEATM to develop the activities that would be undertaken to implement recommendations and to come up with a timeline. ICCVAM and NICEATM have detailed timelines for many of the planned activities, as well as a defined product for each activity, such as a peer review report, recommendations, or validation study. He will provide more details at the next meeting about the planned activities and schedule. Regarding the comment about predicting potential adverse human effects, he said when they look at a new method they look at its performance relative to the traditional method, if it exists. They also look at the new method with regard to any existing human data or experience, which can be accidental exposures as well as ethical human studies. Information from the bioassay is used to help assess the method's ability to identify substances that might cause cancer in people. He said they do have some known human carcinogens are one example where they do have some information with regard to the toxic effects in people.

Dr. McClellan said Dr. Stokes had pinpointed his concern and added that many of the existing methods are not as robust as we would like to portray them. He said by using them as the gold standard, we might come to an erroneous conclusion with regard to new alternative methods; however, if we instead, at the first step, look hard at the alternative method as a predictor of human toxicity, we might in fact come to a different conclusion. Dr. Stokes agreed and referred to the LLNA, reviewed in1998, as being only 85% predictive of the traditional assay in guinea pigs. Fortunately, they had human test data for quite a few chemicals. Both methods had comparable performance for predicting human sensitization potential, and it was on that basis that the LLNA was recommended as a valid substitute for the traditional test. ICCVAM has always requested and considered all available human data in each of its test method evaluations, and will continue to do so for all future evaluations.

Dr. Marsman followed up with a statement about the priorities ICCVAM had laid out. He said he thought the acute toxicity study was an important priority, even though, in many respects, it is an assay whose value is waning. He said many of the things discussed, stakeholder involvement and incentives for participation, are great, but specifically relate to systemic toxicity. He thought it would be more difficult to identify non-animal tests to replace the bioassay and developmental neurotoxicology assays. He suggested starting with addressing what are the relevant outcomes of the acute toxicity studies and how would those be mimicked with non-animal methods.

VII. NATIONAL RESEARCH COUNCIL REPORT: TOXICITY TESTING IN THE 21ST CENTURY

A. Presentation

Dr. Kim Boekelheide provided an overview the NRC's report, *Toxicity Testing in the 21st Century*, which was released on June 12, 2007. He compared where we are now with were we will be some time in the future with the new biology. He stressed that his presentation was his own personal view and not that of the diverse NRC panel, which came from academia, government, industry, animal rights groups, and environmental groups. The charge to the panel came from the EPA. EPA's concern was that the response to address new toxicology issues has been to create a new test to add to the battery of tests. He said that is not sustainable and that our ability to test chemicals is much more limited than the number of chemicals being created. The committee was to be visionary and macroscopic and to address the concerns of coverage, cost, and animal use. He explained the 1983 "Red Book" approach for risk assessment utilizing hazard identification, dose-response assessment, exposure assessment, risk characterization, and risk management. The current approach is animal testing at high doses and extrapolating from those doses down to human environmental exposure ranges. He emphasized that since it was an EPA-driven process, the concern was ambient environmental exposure, not high-dosage exposure such as pharmaceuticals, and how to do risk assessment on those low-level exposures.

The traditional approach has been MTD-driven, high doses, large numbers of animals, low throughput, expensive, time-consuming, and using pathology endpoints. He explained that pathology endpoints are descriptive phenotypic endpoints. Extrapolations are over a large range, doing the testing in the top of the dose range and modeling what the response is likely to be in the human exposure range. He said we apply uncertainty factors for safety, 10 for species, 10 for dose extrapolation, and 10 for susceptible subpopulations, so that we end up with a 100- or a 1000-fold factor. He said it is difficult to determine how well the system that we are using is working. He described a review of concordance of toxicity of 150 compounds in humans and animals that showed a true positive human toxicity concordance of 71%. The concordance varied a lot among different tissues and those differences were not explained by differences in metabolism.

Dr. Boekelheide said the panel worked on the report for two and a half years through ten meetings. The report is very macroscopic and generated a very big picture. He presented the committee roster, described their diverse background, and identified Dr. Daniel Krewski as the chair. He said the critical decision that the panel made was to move toward a new phenomenon they described as "toxicity pathways," to be the focus for how to develop knowledge about response. The concept is very parallel to cancer studies in that toxicity pathways would be like oncogene and tumor suppressor-type pathways. On the toxicity side, there would be protectant pathways, e.g., heat shock protein responses or apoptotic pathways. The concept is to go from a molecular explanation of the mode of action of response to an exposure within cells to try to understand mechanism, rather than phenotypic responses. The whole paradigm is developed around the concept of toxicity pathways. He said we have been looking at oncogenes and tumor suppressors since the war on cancer began 35 years ago. We have made substantial progress and can now robustly predict the behavior of a cancer based on its oncogene and tumor suppressor expression, in terms of forecasting how the cancer will behave, on par with, and independently predictive of, pathology endpoints. He said it took 35 years to develop that database and the panel envisions it would take 25 to 50 years to change the paradigm for the way in which we do testing.

He described a response pathway in which at a very low dose, there will be disruption, but the pathway will continue to function, due to the homeostatic response. At a higher dose there may be adverse or adaptive responses that take place, cellular changes; they are then responded to, and the cell can adapt and the pathway can continue to function. At a higher dose yet, there may be some kind of irreversible change in the pathway function that leads to injury to the cell or organism that has severe consequences and is therefore no longer adaptive, but adverse.

He reviewed components of the report's vision, which include (1) chemical characterization, (2) dose-response and extrapolation modeling, (3) toxicity pathways and targeted testing, (4) population and exposure data, and (5) risk contexts. He summarized the steps of risk characterization, starting with chemical characterization and ending with the creation of exposure guidelines.

The implementation of this strategy involves (1) a comprehensive suite of *in vitro* tests, preferably based on human cells, cell lines, or components; (2) computational models of toxicity pathways to support application of *in vitro* test results in risk assessments; (3) infrastructure changes to support basic and applied research needed to develop the tests and pathway models; (4) validation of tests and test strategies; (5) and evidence justifying that the toxicity pathway approach is adequately predictive of adverse health outcomes to use in decision-making.

Dr. Boekelheide stated that this strategy promises human relevance, dose relevance, chemical coverage, a mechanistic focus based on mechanism/mode of action, is cost effective, fast and will result in fulfillment of the 3Rs. However, conundrums exist regarding screening tool vs. stand-alone test, validation, the abnormal biology of human cell lines, mixtures, metabolism, epigenetics, cell-cell/organ interactions, adaptive vs. adverse responses, the over-promise of toxicogenomics, use of unfamiliar surrogates, the issue of this being another "war on cancer," and "who is going to do this and where is the money going to come from." He said he thinks it should be done in some independent, newly established, institute setting, modeled after the Human Genome Institute, and it will cost a lot of money (the panel estimated $300M/year over 20-plus years).

He said the knowledge from animal studies can be used to better understand the molecular pathways and interpret them as they apply to people, using information from people to interpret them correctly. He said he thinks this a different way of conceiving how the work will be done compared to the proposal described in the Collins, Gray, and Bucher article (Science 319:906-07), which uses the information at the molecular level to prioritize the animal tests. The NRC proposal is ultimately meant to be a stand-alone paradigm for testing.

B. SACATM Discussion

Dr. McClellan commented that most scientists love "discovery science, discovery research," and are not happy with "issue-resolving science." This is issue-resolving science that builds on discovery science. Dr. Boekelheide agreed, saying that it is applied. Dr. McClellan said some scientists might be concerned that funding will be applied to this effort and not to their grants. Dr. Hamernik asked about the statement that most toxicological data are from high dose testing. Dr. Boekelheide said the panel based this statement on MTD requirement and extrapolation, which are orders of magnitude from the environmental exposure. It is done on purpose due to

time constraints to get a response and is based on the NTP model. Dr. Hamernik said there are other types of toxicity testing designs, such as the pesticide program at EPA, where they don't do just carcinogenicity testing. Dr. Boekelheide replied that they are still several orders of magnitude above environmental exposures.

Dr. Marsman asked if the panel felt limited by the constraints of looking at environmental exposures. He struggled with the focus at the low dose, because in reality, few in industry have the luxury of just doing the risk assessment from that low dose exposure. Industry deals with their products from the standpoint of the raw material supplier, the high doses in the plant, transport to the manufacturing site, environmental exposures in the effluents from the plant, disposal of the product after it's used and CPSC and everything related to its use while in commerce. So the exposures have a wide range. Dr. Boekelheide responded that the panel considered dose-response within the paradigm of the toxicity pathway, which should be robust across a wide dose-response. Dr. Brown asked about using this approach in a pharmaceutical environment. Dr. Boekelheide said the paradigm should work, even at those levels.

Dr. DeGeorge asked why the panel moved from a phenotypic/observational endpoint approach to a mode of action approach. Dr. Boekelheide answered that if we know about mechanisms, then a lot is known about mixtures and we can understand interactions between compounds better. He used the cancer analogy, likening grading tumors to predicting outcome, to using molecular pathways to predict outcomes, i.e., looking at fundamental properties of effect as opposed to measures of effect that are not fundamental to how chemicals interact with tissue.

Dr. Ehrich, a lead discussant, provided written comments, which Dr. White read into the record: "I have read the entire report and used it for teaching my graduate course (Pharmacology & Toxicology Testing, Virginia Tech BVMS 5214) Spring semester 2008. On the plus side of the equation, the report provides something that is innovative and visionary. (1) On the other side, one needs to consider the time needed to reach even early milestones toward the destination. Also, integrative system toxicities (e.g., neurotoxicity, toxicities related to changes in endocrine or metabolic systems) are likely to be very, very difficult without the use of whole animals. Much development is needed and the research for this development will be a big need. (2) A role for ICCVAM/NICEATM would be to support new test development. (3) ICCVAM/NICEATM's role in changing regulations would require good validation studies. As these studies are being done, the agencies would need to be kept informed."

Dr. Barile, a lead discussant, said the report could be looked at as either taking a step backwards or reevaluating toxicity information that was done years ago before the development of *in vitro* tests, i.e., look at mechanistic toxicology. The idea of developing *in vitro* tests in the 90s was to develop quick, short-term tests to go along with the mechanistic studies, which is where the idea of basal cytotoxicity came from. He said cell tests and minimal mechanistic target pathways could be used to explain toxic effects that are common to all cells. The advantage of the mechanistic pathway suggested in this report is, if you can find a particular mechanism or target-like organ in the cell cultures, it might help in developing either short-term tests or a stand-alone test or screening test; however, he added it is not clear how that would work. It will take another 25 to 30 years to develop screening or stand-alone tests based on mechanistic information. He added that there would not likely be many developments in the 3Rs within the next five to ten

years based on this report and that is important to keep in mind. The mechanistic tests have a lot of information that will be derived, e.g., specificity and sensitivity. The disadvantage is that they are not short-term tests. The advantage is that mechanisms can aid our understanding of the site of action. He said we would have to be patient to see how the bridge is gapped between mechanistic information, toxicity testing data, and risk analysis. He said the report gives some information on how to form a new institute. As an academic, he doesn't believe too much in the value of forming large organizations. Instead of a new institute, he suggested expanding ICCVAM-NICEATM. Formation of a new institute means money and resources, all of which are currently limiting. Taking the existing institutes and giving them more funding, resources, and administrative clout would be a better way of using resources. ICCVAM-NICEATM have experience in knowing how to bridge the gaps between toxicity testing data generated from these undeveloped tests toward the idea of risk assessment. He said we have enough mechanistic information and biotechnology that could be used for the development of new tests, e.g., HTS tests, microarrays, PCR. The information that is already generated could be used as a screening test for target-oriented mechanisms.

Dr. DeGeorge, a lead discussant, agreed with Dr. Barile and thought that 20 to 50 years is a realistic estimate for going the further step from the microscopic to the "nanoscopic" and looking at the biochemical mechanism, but in 20 years, cell-based assays could be done. The advantage of the mechanistic approach is discovery of widespread, fundamental mechanisms of toxicity. Hundreds of millions of dollars have been spent for a decade or two to measure apoptosis correctly and distinguish it correctly from other types of cell death. People will need to be trained in those technologies. He said we might discover fundamental principals that would hopefully explain in the existing classical toxicology tests the discordant, accepted, low accuracy of some toxicology tests when compared to an alternative or human data. Guinea pig data compared to rodent data yielded a high 80% concordance accuracy. When each one is compared to the human data the accuracy is in the 72 to 74% range. The advantage of proceeding toward mechanistic toxicology is that it will allow us to resolve conundrums from the past, but will also bring on a whole new set. He agreed that ICCVAM needs more staff and resources. He said it is important to remember that there are 3Rs and that it is not necessary to go for total replacement of animal testing. The gave the example of the 3T3 phototoxicity test. He suggested a conservative approach and not moving to high throughput too soon.

Dr. Fox, a lead discussant, lauded Dr. Boekelheide and the NRC committee for the report and added that most toxicity is not like cancer. Neurotoxicity and reproductive and developmental toxicity cannot be modeled the same way that a hyperplastic response can be modeled. A single endpoint is looked at in cancer and with all other systems, there are multiple endpoints and the tissues are complex. He expressed concern that the committee chose to use the cancer model as the global model on which to base their thought processes. He said the report was brilliant and well-written and reflected a lot of careful thought, but that they left out some of the most important people, cell biologists, systems biologists, and people who think from the top down and from bottom up at the same time. He mentioned the pathway assist programs and the use of curated pathways, giving examples from his own laboratory. Dr. Fox expressed concern about reversibility and the fact that in HTS systems, reversibility is not detectable. He said this is critical and pointed to the discussion of adaptive vs. adverse. Certainly there is adaptation, but reversibility is a big phenomenon as well. He said you can actually find cells that undergo a

proliferative response following an insult, e.g., a heat shock response that is toxic, but it's short-term and reversible. He mentioned delayed effects from exposures in early development that don't manifest themselves until 6, 12, or 20 months of age in animals and until the 5th, 6th, 7th, or 8th decade of life in humans. He asked how that would be assessed in a cell. He said he was not trying to be overly negative, just a good scientist pointing out the disadvantages. Maternal-fetal effects could be considered cell-cell interactions. Regarding sensory, motor, and cognitive effects, he was unsure how this might be approached in this paradigm. He asked how aging and susceptible populations are identified in cells and how the cell systems would be chosen. He said Dr. Boekelheide made a good point about using human cells, that most of them are transformed. He referred to the Science paper mentioned earlier stating that about 80% of the cells are transformed cell lines, and said we know that neuroblastomas don't respond normally adding that some do not even have the same complement of signaling pathways, especially the cytokines. He brought up epigenetics and substrates and noted that what the cells are grown on can influence the results. He asked about data analysis in terms of the hundreds of thousands of runs per day, corrections for multiple samples being run at the same time, *p*-values, and multiple corrections. He said he thought that the tiered testing approach is good and that it should be incorporated into the report. He said the strength of the report lies in the impact on regulatory decisions. Coming up with rational approaches will allow risk assessments to be done. They have provided a great approach, but other decisions have to be made. He said the effort should all be at NIEHS, that a new institute is not needed. The extramural program at NIEHS should be involved and interact with the intramural scientists. He said ICCVAM should be involved and the process should include all stakeholders. He thought the cost would be high, at least a $10 billion project. He acknowledged that NIEHS is under-funded, but considers this project important to protect human health.

Dr. McClellan complimented the authors of the report and Dr. Boekelheide for his presentation, stating that it was appropriately nuanced. He expressed concern that the report does not adequately recognize the range of human disease. It used cancer as a prototypical model, which he considered a serious mistake, possibly because many people on the panel were trained in that approach. He referred to national disease statistics showing that cancer, which is a very diverse family of diseases, is down the list and cardiovascular disease is higher on the list. A strength of the report is that it is visionary, but it lacks the broader view necessary to understand that humans are afflicted with a broad array of diseases. He said it is almost mind boggling to think of all of the pathways and interactions that can occur with regard to disease. He said the report lacks a strategic implementation plan. He viewed the FYP and NRC plans as having some overlap and suggested that ICCVAM-NICEATM can facilitate that process by continuing to encourage the institutional structure to push ahead with trying to create an implementation plan for the NRC approach. As ICCVAM sharpens its implementation plan and its strategy, it should consider giving greater emphasis to a disease orientation. ICCVAM needs to develop that broader view of variability across populations, because it is going to drive many risk assessment approaches in the future. The extent to which ICCVAM sharpens its validation methods and links them to known human toxicities will provide a template for approaches advocated within the NRC report.

Dr. Dong asked about the NRC approach to first use animal cells as an option, and the ethical issues of continually getting human cells, especially from healthy humans. Dr. Fox responded

that the cell lines are transformed and very few primary cells lines are involved, so access to tissue is not an issue. Dr. Bucher said in NTP's initial investigations at NCGC, they have used both animal and human cells from corresponding organs and unfortunately sometimes the responses have differed. This is an area currently under investigation. Dr. Fox said there are two cells lines being used in the neurotoxicology field and one is much more sensitive than the other, which raises the question about which to use as the sentinel because it will make a difference in evaluating the dose-response curves.

Dr. Brown said one advantage of this approach is the potential of helping us refine animal studies. We can learn more about mechanisms and what types of cells or organs are targeted and focus our observations and develop earlier endpoints. She expressed concern about the potential for false positives because it seems that this approach looks at toxicology as an event rather than a process. She referred to the comment regarding reversibility and added that if you just look for a specific reaction it may not be representative of what would really happen in the whole organism.

Dr. Charles added that it would take a new breed of scientists, somewhat comfortable with *in vitro* test systems, and with modeling systemic biology, to put this approach into effect. The same is also true in a regulatory context; the staff interpreting these data has to be educated about how to interpret them. It will have a significant impact on regulatory decision-making and education will be key, not just for the toxicologists performing the tests, but also for the regulators. He agreed with Dr. Fox, that it should be a single agency-driven approach. In terms of ICCVAM-NICEATM serving and implementing this vision and this strategy, he saw it as an iterative process, where the test systems are developed and utilized to generate this conceptual basis for looking at the toxicity pathways. In terms of when they go into effect for developing new products or chemicals, he saw ICCVAM as helping to implement the validation of the methodologies.

Dr. DeGeorge reiterated that a lot of progress has been made in cell biology. He said he thinks the ultimate goal is to understand how human cells behave, how they function normally or abnormally, at the cellular level (paracrine, autocrine), at the intracellular level, all the way down to the intranuclear. He said we should not throw away time and effort with already established nonhuman cell types, citing the example of using 3T3 cells in a photobiology study using 96-well plates to assay viability, apoptosis, or necrosis to generate mechanistic data. He said data are being generated by tests now in that cell line that can be explored mechanistically. He said his main point is not to rush to use human cells. He said we have 50 years of knowing how a lot of different cell lines respond and they have been fully genotyped and suggested capitalizing on that knowledge. He said it is harder to work on human cells, whether transformed or primary cells. He said we need a stepwise plan using rodents or whatever intermediates are available. He estimated that the project might only cost $8 billion, rather than $10 billion.

Dr. Freeman summarized the discussion by saying that ICCVAM had received a range of commentary from scientific to practical to, "don't throw the baby out with the bathwater." Dr. Stokes thanked the panel for the comments and said as they work toward an implementation plan for the ICCVAM-NICEATM FYP, they would be considering the implementation strategy

and plan that will be brought forward for the NRC report. He said there is overlap and they need to take advantage of that overlap.

VIII. FEDERAL AGENCY RESEARCH, DEVELOPMENT, TRANSLATION, AND VALIDATION ACTIVITIES RELEVANT TO THE FIVE-YEAR PLAN

A. Presentations

1. National Institutes of Heath

Dr. Norka Ruiz Bravo said the NCR report provided a perfect prelude to her presentation and she congratulated the NCR committee for having a vision for the next 20 or 30 years. She said the important point about having a vision is not so much the details, because they're not there yet, but it forces us to think beyond where we are already are and about the things we could do.

She reviewed the mission of NIH, which is stewardship of "medical and behavioral research for the Nation. Its mission is science in pursuit of fundamental knowledge about the nature and behavior of living systems and the application of that knowledge to extend healthy life and reduce the burdens of illness and disability." NIH is interested in the pursuit of fundamental knowledge and the underpinnings of what will inform application of the knowledge. She explained the four Ps of biomedicine in the 21st century: predictive, personalized, preemptive, and participatory. She said NIH supports using the best models for the science in question and she mentioned some evolving public health challenges including: (1) a shift from acute to chronic conditions, (2) aging populations, (3) health disparities, (4) emerging and re-emerging infectious diseases, (5) emerging non-communicable diseases, and (6) biodefense.

Dr. Ruiz Bravo explained the dual nature of NIH in that it both does research and supports research at other institutions. She reviewed the organizational structure and explained that the total FY 2008 enacted budget is $29.457 billion, 53% of which is for research project grants and 10% for intramural research. She mentioned ICCVAM's mission and the FYP priorities and said that NIH fits into the FYP priority of incorporating new science and technology. She asked, "Can the biomedical research supported by NIH lead to the development and validation of alternative regulatory safety tests?" Dr. Ruiz Bravo said NIH science and technology are developed to understand biological systems and promote human health and that NIH-supported research may open new possibilities for alternative toxicology tests.

She showed a schematic of the NIH Roadmap for Medial Research, which provides a vision for NIH. It has a number of projects that will be relevant to toxicology testing. It is very fundamental research that may be useful for future applications.

Dr. Ruiz Bravo provided some examples from NIH's research portfolio:

> Zebrafish are being used as a model system in toxicology studies because they are small, have a short lifespan, are very fecund, are optically transparency, have genetic tractability, and are ectothermic.

The NIH NCGC is part of a collaboration between NIEHS/NTP and EPA to create a national resource in chemical probe development. The point is to use chemicals that are known to have a toxic effect to understand why they are toxic, what genes they affect, and the mechanism by which they have that effect. That information is then applied to large numbers of chemicals.

Genomics-based nosology, a new way to classify diseases genetically, provides a way to look at the underpinnings of disease and find disease commonalities using gene chips and microarrays. This is being called the "disease-ome," which is the collection of all the diseases and the genes that are associated with them.

Three-dimensional (3-D) tissue modeling goes beyond two-dimensional modeling that cannot mimic *in situ* complexity. 3-D data are more physiologically relevant for cell-matrix interactions, cell polarity and barrier functions, spatial/temporal gradients, the role of biomechanics, and mixed populations in relevant configurations. Advantages of 3-D respiratory tissue models include being able to look at developmental biology, alveolar gas exchange, the role of stress, and real time monitoring for studying disease progression. Common building blocks are used in generalized 3-D tissue models. A 3-D liver metastasis model has been used to demonstrate the feasibility of this approach using a microscale perfusion system and growing a solid tumor. Dr. Ruiz Bravo mentioned two recent publications, Collins *et al*., (2008) and the FYP, that highlighted the need to examine and integrate HTS and the use of *in vitro* systems to screen environmental toxicants. She said the 3-D models could complement these methods at many levels. The ultimate outcome will be more predictive models of human responses that incorporate pharmacogenomics as well and reduction and refinement in our use of animals in biomedical research. There is a clear opportunity here to transform and accelerate drug discovery.

NIH Small Business Innovation Research (SBIR) Program is a set-aside for innovative development of projects that are translational. The Mattek EpiAirway System is a 3-D human airway model used as a drug screening and research tool for cancer drugs. The company has a SBIR grant to validate it as a model for toxicology applications.

Dr. Ruiz Bravo said, in closing, that she did not want to over-promise and that NIH supports fundamental research that may be years from being applicable to HTS kinds of screens that toxicologists would use. She said the promise is there, and that it is worth thinking about and exploring. She applauded the NRC for articulating a vision. She said we need to capitalize on the new technology and new science and if we do not we will be left behind. She said these are concepts, a vision, and not a finished proposal. She suggested thinking hard about what the direction is going to be, what the low hanging fruit in the short term might be, and if there is anything where we could emphasize translation. She said, in the long term, we need to think about where we need to be in the next five, 10, 15, or 20 years with this project.

SACATM Comments

Dr. Fox asked if the 3-D tissue models were distinct from tissue slices and if tissue slices have an equal or parallel place in the process. Dr. Ruiz Bravo said she would not want to close the door on anything that works and with tissue slices we should ask what the limitations are. She added that 3-D models will be part of the NIH Roadmap and there will be a solicitation for innovative, transformative research.

2. National Institute of Environmental Health Sciences/National Toxicology Program

Dr. Bucher said the NTP has four goals, two of which relate to this discussion: (1) strengthen the science base in toxicology and (2) develop and validate improved test methods. He said NTP has been in existence for 30 years and has looked at a large number of chemicals and endpoints, an enormous amount of work, but not a sustainable effort to meet the future needs of toxicology. Recognizing this a number of years ago, we developed the NTP Vision for the 21st Century, which emphasized moving from observational science to more predictive science based on mechanism-based biological observations. In the 2004 NTP Roadmap, three of the elements relate specifically to this discussion: (1) further evaluate and refine the use of non-mammalian animal models, (2) develop HTS capabilities for assessing mechanistic targets *in vitro*, and (3) create analytic capabilities to integrate diverse toxicology information to add value and understanding.

He alluded to the excellent presentation by Dr. Boekelheide on the NRC Report and mentioned the NRC report *Applications of Toxicogenomic Technologies to Predictive Toxicology and Risk Assessment*. Both documents had the same goal, which is to begin to articulate the ways in which information from high-density data developed through HTS and genomic technologies can be brought into the risk assessment realm.

The NTP Roadmap has a major initiative to develop a HTS program with three goals: (1) identify mechanisms of action, (2) develop predictive models for *in vivo* biological response, and (3) prioritize substances for further in-depth toxicological evaluation. In 2005 we began a partnership with the NIH Molecular Libraries Initiative (MLI) and the NCGC to identify batteries of cell-based and biochemical assays to probe toxicity pathways. NTP has supplied chemicals, assays, and financial support to the NCGC and is developing tools to link data generated from HTS assays to data produced by the NTP toxicology testing program. The current focus is on toxicity pathways in immune function and cancer. Toxicity pathways are common to many diseases and NTP has a large animal database related to the cancer outcomes and a large amount of data from rodent immunotoxicity studies as well as information about the processes involved in immune function and disease in humans.

In 2007 NTP created the Biomolecular Screening Branch, headed by Dr. Ray Tice, Acting Branch Chief, to develop the research and testing activities in high and medium-throughput screening for rapid detection of biological activities of significance to toxicology and carcinogenesis. NTP is developing analysis tools and approaches to allow an integrated assessment of HTS endpoints and associations with findings from traditional toxicology and cancer models. They are also carrying out the NTP automated screening assays with *C. elegans*. Recruitment for a permanent branch chief and bioinformaticists is currently underway. The first

chemical set was screened in most of the 96 assays currently at NCGC and an additional 4000 chemicals are being selected.

Targets for toxicity pathways are being identified. NTP has provided a number of the 96 NCGC assays that are currently being used. NCGC funding has been increased and recently a memorandum of understanding (MOU, *High-Throughput Screening, Toxicity Pathway Profiling and Biological Interpretation of Findings*) with EPA and NCGC was signed. Dr. Zerhouni is very interested in developing the activities under the MOU. Dr. Bucher said move to forward, it must be done in concert with the MOU partners and explained what each of the partners is bringing to the MOU. NTP brings historical toxicology data and experimental toxicology capabilities to analyze in standard animal models the output from the HTS models. The structure allows iterative testing and is a way to understand the HTS output. There is a niche for the lower organism models, *C. elegans* and zebrafish. The *Science* paper and the NRC report call for a targeted testing program that allows movement from the very lowest level of cell-based assays, up through an intermediate level, before getting to mammalian systems. Computational toxicology is covered and there is some effort to begin to look at the influence of genetic background using defined cell lines that differ in certain genetic properties. ICCVAM-NICEATM brings a wealth of validation experience.

Dr. Bucher explained that the HTS activity is being carried out using four working groups: pathways/assays, compounds, bioinformatics, and targeted testing. Of the universe of 13,116 chemicals in common commerce, approximately 7000 are relevant to NTP and EPA. He explained that EPA's ToxCast™ program has a number of contracts for generating HTS and genomics data. They have a program including over 400 different assays using 300 chemicals and there will be an attempt to integrate HTS and selected genomics data. ToxCast™ is currently in phase I; phase III will consist of the prediction and prioritization of thousands of chemicals by FY 11-12. He also described WormTox, which uses *C. elegans* for mid-throughput toxicological screening of developmental and neurological toxicants.

Dr. Bucher explained development of the Chemical Evaluation in Biological Systems (CEBS) Knowledgebase, which will be used to integrate information from all the databases at NIEHS. He briefly mentioned the 3-D tissue model component of the NIH Roadmap. He stated that the Collins *et al*. (2008) paper was to lay out a logical way to address the challenges in the NRC report. He described a NTP 21st Century Model wherein toxicants are initially examined using cell-based HTS technology, then tested in model organisms (*C. elegans*, zebrafish), then considered for mammalian "in life" studies, and finally uses an iterative process to develop predictive models. This will allow information from *in vitro* studies to be used to design better animal studies.

SACATM Comments

Dr. McClellan said he was pleased to see 100 human toxicants identified in the ToxCast™ program and asked if the compounds are present within the NCGC, and Dr. Bucher said they are. Dr. McClellan asked about the array of diseases represented by the 100 compounds, because he thought that was very important that the chemicals be selected carefully. Dr. Fox said the iterative process was systems biology, the "Leroy Hood definition." He referred to the six

known pathways surrounding known toxicants and said they interact in a Venn diagram-like way with human disease. He said this is the tiered-testing approach that was not present in the original document. In response to a question about resources from Dr. McClellan, Dr. Bucher said they put about half of their man-hours into the toxicology program in terms of assay miniaturization and running the assays.

3. Environmental Protection Agency (EPA)

Dr. Hamernik noted that EPA and ICCVAM have had an active partnership since the year of ICCVAM's inception in 1994 and that the EPA has benefited from the information and analyses provided by ICCVAM. Previous and ongoing collaborations include evaluation of guidance and validation issues, development of performance standards for alternative test methods, interactions with the OECD test guidelines program, ICCVAM workshop/workgroup participation, and follow-up on EPA nominations. She highlighted some recent interactions such as those involving the Local Lymph Node Assay and eye toxicity classification and labeling. She went on to mention Agency support for other initiatives related to the 3Rs such as for an ILAR/NAS humane care project and for Agency participation in activities related to the Center for Alternatives to Animal Testing (CAAT).

She enthusiastically announced that an Agency-wide Future of Toxicity Testing Workgroup (FTTW) had been established to develop a response to recommendations in the 2007 NRC report on Toxicity Testing in the 21st Century. The Workgroup produced a strategic plan for the future of toxicity testing at EPA that would take on the challenges presented in the NRC "vision" document.

Components of the FTTW plan include a number of strategic goals such as toxicity pathway identification, chemical screening and prioritization, toxicity pathway-based risk assessment, and institutional transition. The strategy discusses utilization of computational toxicology approaches such as ToxCast™. The plan is a critical initial step in a long-range process to move forward with a strategy for a toxicity testing paradigm shift at EPA. She mentioned the MOU between EPA, NTP and NCGC to leverage resources and expertise and strengthen collaborations on high throughput screening (HTS), toxicity pathway profiling, and biological interpretation of findings.

She then went on to describe an Agency research initiative to develop a new approach to address information gaps for chemical hazard and risk assessment for EPA program needs using computational approaches such as ToxCast™. ToxCast™ uses a variety of HTS assays/techniques to derive chemical profiles (signatures) for hundreds of endpoints with potential relevancy for carcinogenicity, reproductive toxicity, developmental toxicity, neurotoxicity, and chronic toxicity. She described the envisioned multi-phased development of the ToxCast™ program (note information presented could change depending on circumstances), starting with ~300 relatively data rich pesticides, with the ultimate goal of assessing thousands of chemicals having less available data by around FY09-12, at lesser cost. She described a number of databases proposed for use for information management. There is also ongoing development of virtual tissues, organs and systems (e.g., the Virtual Liver Project), with the goal of linking exposure, dosimetry and response to predict potential effects.

Dr. Hamernik further discussed the goal of integrating various types of information to identify toxicity pathways that, when sufficiently perturbed, are expected to result in adverse health effects. She discussed moving forward with "the vision" and the inevitable challenges in development, implementation, integration and acceptance. She quoted the NRC report: "despite the established value of *in vitro* systems . . . increased reliance on them for regulatory testing may require further evidence of validity." She provided some perspectives on internal and external validation as applied to the development of methods. She ended with some thoughts on substantiating prediction chains at progressive levels and gave as an example the progression of information levels starting from "omics" technologies up to that of relevance for human health or the environment.

SACATM Comments

Dr. McClellan, a SACATM member, had a question about the ToxCast™ program. He made reference to "100 human toxicants" and wanted to know how these fit into the ToxCast™ program. He was also concerned that the program might use *in vitro* tests that had not been validated and/or that validation efforts would use rodent, not human data. There appeared to be some confusion about what chemicals were being referenced as the "100 human toxicants". Dr. Hamernik asked for the question in writing and said she would provide an answer after receiving it, although she indicated that might not be possible until after the meeting. She mentioned that ToxCast™ was discussed as an Agency research and development program. Dr. McClellan said he would provide the question in writing and requested that it and the response be put in the record. Following the meeting and for the record, Dr. Hamernik provided a detailed response from the EPA to Dr. McClellan's question which explained the U.S. EPA's multi-phased ToxCast™ research program more fully and addressed the issue of the "100 human toxicants" mentioned by Dr. McClellan. [Appendix A]

4. Food and Drug Administration/National Center for Toxicological Research (NCTR)

Dr. Howard reviewed the FDA mission to protect the public health by assuring safe and effective medical products and safe foods for humans and animals. He said the FDA regulates about 25% of the commerce in the United States and is divided into centers, each regulating products under specific federal laws and statutes. In 2004 there was the realization that the time it takes drugs to get from concept to approval by FDA is too long, so they came up with the Critical Path Initiative to modernize the way in which FDA-regulated products are developed, evaluated, and manufactured. In 2006 FDA created the Critical Path Opportunities list, which describes the accomplishments and opportunities that could help speed development and approval of medical products. It is broken into six areas: better evaluation tools, streamlining clinical trials, harnessing bioinformatics, moving manufacturing into the 21st century, developing products to address urgent public health needs, and identifying specific at-risk populations such as pediatrics.

Dr. Howard reviewed NCTR's mission and listed their strategic goals: (1) advance scientific approaches and tools to attain personalized nutrition and medicine; (2) develop science-based best practice standards and tools to incorporate translational and applied toxicological advancements into the regulatory science process; and (3) develop and apply rapid detection technologies and testing platforms to assure food safety, biosecurity, and food biodefense and to

combat terrorism. He described the four ways that an NCTR investigator initiates a project: investigator-initiated; provide data on specific material to support an FDA Center's risk identification or risk assessment; FDA-specified area of need; and interagency requests for research expertise.

He said there is key overlap between FDA's mission and two of the four key challenges identified by ICCVAM in the FYP (identifying priorities and conducting and facilitating alternative test method activities and incorporating new science and technology). He listed some of the research projects at NCTR: genotoxicity, mutagenicity and exposure biomarkers of acrylamide; DNA adducts of Tamoxifen; toxicities of antiretroviral drugs; food-borne toxin potencies; assays for ricin; chemical inactivation of protein toxins on food-contact surfaces; immunogenicity of permanent makeup inks using a modified LLNA; DNA adduct formation, mutations and patterns of expression in Big Blue rats; epigenetic mechanisms of cancer; manganese nanoparticle neurotoxicity in PC12 cells; and "Mitochip" assays. They are looking at assays that have the potential for being *in vitro* assays predictive of animal models.

He described the talk given by Dr. James Fuscoe titled, *Impact of Systems Toxicology on the 3Rs," at the 6th World Congress*. It delineated what is needed for genomics and the Microarray Quality Control Project, which started at NCTR. It feeds into FDA's Voluntary Genomic Data Submission program. He said Dr. Fuscoe thought that metabolomics, genomics, and proteomics would be good tools to apply toward refinement, reduction, and replacement of animals. He added that many technologies used at NCTR, such as assays for DNA methylation, epigenetics, single nucleotide polymorphisms, and alternative tests, are useful for the 3Rs as well. He concluded by saying that through fulfilling its role in the FDA and Critical Path, NCTR is addressing some components of two of the four key challenges for ICCVAM in 2008-2012.

SACATM Comments

Dr. McClellan asked about the success of voluntary submissions. Dr. Howard said he thought there had been good participation from the drug companies and that it has been quite successful in terms of allowing FDA to look at the data and have informal discussions with industry.

5. Food and Drug Administration/Center for Biologics Evaluation and Research (CBER)

Dr. McFarland stated the mission of CBER and noted that CBER's and ICCVAM's missions are complementary. CBER has three product offices that regulate: (1) blood and blood components, (2) vaccines, and (3) cellular, tissue, and gene therapy. He explained that the human biologics regulations are typically focused on endpoints as opposed to requiring specific tests. This facilitates CBER's acceptance of data from innovative and varied non-clinical testing methods if they are shown to provide data that are useful in product assessment. CBER uses this approach in its routine individual discussions with sponsors to further the principles of the 3Rs while protecting the public health.

He highlighted CBER research priorities, selected intramural research activities relevant to the 3Rs, and how the CBER "researcher-regulator," provides the agency a research expertise in areas not covered by academia or industry. Relevant CBER FY08 research priorities are: improve or

develop new methods to measure and augment biological product safety and efficacy; evaluate, develop, and integrate novel scientific technologies; and facilitate the development of new biological products.

He described work on the development of "detector cell lines" for rapid evaluation of activity and safety of novel adjuvants. This work has potential benefits by predicting *in vivo* toxicity and reducing the need for animal testing. Stage I is to evaluate adjuvants that are licensed or tested in clinical trials. He showed data for differential pro-inflammatory cytokine responses in MM6 cells treated with various adjuvants and delivery systems. They are looking at other cell lines to assess potential neurotoxicity and hepatotoxicity.

Another line of research is the *in vitro* measurement of vaccine potency for a vaccine to botulinum toxin. This work uses an ELISA to quantify vaccine antigenicity relative to native neurotoxin as a potential approach to developing more effective vaccines. He showed preliminary data that suggest protective immunity *in vivo*, comparing a commercial toxoid and CBER-developed toxoids. This method could potentially be broadened to develop similar ELISAs for other vaccine candidates.

He also described a FDA project with NTP for the development of pre-clinical assays to evaluate the cancer risk of new and existing gene therapy products. He said when the data are available; they will be shared with researchers in the field so that they will be able to incorporate what was learned from this study into subsequent product development. In conjunction with this study they are also developing genotoxicity assessment in the replating assay, which is an *in vitro* tool to screen for tumor risk. The study will include side-by-side assessment of vectors using the *in vivo* and *in vitro* assays to determine the sensitivities and reproducibilities of both methods. These studies may allow investigation of newly developed vectors with different promoters to minimize the risk of tumorigenicity.

Dr. McFarland also described a sampling of initial work in the development of pre-clinical assays for characterization of cellular therapy products. CBER and collaborators are using *in vitro* techniques (flow cytometric and "omic" technologies) to identify potential biomarkers for mesenchymal stem cell quality and biomarkers that correlate with self-renewal or differentiation. He concluded by saying that CBER's research program addresses the ICCVAM priorities in the areas of biologics, immunotoxicity, and chronic toxicity/carcinogenicity and thus relate to all four of the FYP challenges.

SACATM Comments

Dr. Barile asked how CBER prioritizes its intramural research. Dr. McFarland answered that priority-setting is an annual activity involving public health concerns, agency mission, and resources. These priorities are peer reviewed within the center and with the FDA Science Board and the appropriate FDA advisory committees.

6. National Cancer Institute (NCI)

Dr. Tomaszewski said NCI has for the past 10 to 15 years been moving away from the use of animals to develop *in vitro* assays, with the hope of moving to HTS assays and *in silico* methodologies to develop cancer drugs. Toxicity testing is important at NCI because they want to move things from the bench to the bedside as rapidly as possible.

He explained that the Cancer Chemotherapy National Service Center (CCNSC), the predecessor of the Developmental Therapeutics Program (DTP), was created in 1955. He described the organization structure of DTP, which has a discovery and a development component, and the chronological change in DTP responsibilities. He reviewed the drug development programs supported by DTP. More recently they have become almost like a CRO for NIH in terms of drug development. They are currently developing the NCI Chemical Biology Consortium to leverage all the resources in the NIH Roadmap. The Division of Cancer Prevention has some drug development programs utilizing biomarkers, pharmacokinetics, pharmacodynamics, and toxicology. The NCI Nanotechnology Characterization Laboratory is doing both *in vivo* and *in vitro* studies.

Dr. Tomaszewski stated that the prediction of human toxicity and sensitivity in drug development is important because cancer drugs are some of the most toxic compounds purposely administered to human. It is necessary to predict a safe starting dose, MTD, and dose-limiting toxicities. He outlined the FDA requirements for preclinical pharmacology and toxicology in assessment of small molecules and biologics and stressed that the study designs are agent-directed and not done simply to check a regulatory box. He said the main causes of failure in the clinic include safety problems and lack of effectiveness. In oncology, only 5% of the agents that get into phase I are approved.

Regarding animal data being sufficient to predict human toxicity, he stated that dog and primate are more predictive than other species; however, a high degree of safety is possible using toxicity data from rodents and non-rodents, but in estimating MTD, dog > mouse > rat. For dose-limiting toxicity, the dog does best as well; however, toxicities are not predicted well, except for bone marrow and GI. Regarding *in vitro* toxicity data increasing the safety margin in the clinic, in the *in vitro* bone marrow assay, *in vitro* data are comparable to dog data. He presented data from studies comparing Topotecan vs. Indenoisoquinolines using human vs. mouse bone marrow and ECVAM studies predicting human MTDs.

Currently, NCI is doing additional development and validation of *in vitro* assays using lung and liver slices and assessing cytokine responses following administration of the aminoflavone prodrug. Future studies include *in silico* and HTS evaluations to do screening work and iterative chemistry. He closed by discussing the evolution of DTB in drug discovery and development and stated that to validate *in vitro* or *in silico* assays, both animal and human data are required. Cancer drugs are ideal for this purpose since there is a wealth of both animal and human data.

SACATM Comments

Dr. McClellan asked what drugs have gone through the NCGC program. Dr. Tomaszewski answered that what NCI was doing with NCGC is very different. They are using drugs right now primarily for target assay development for activity and efficacy and will test them in toxicity

assays at a later time. Dr. Tomaszewski said they have not discussed the different types of assays that would be utilized and the different agents. They would have to work out an agreement with the companies. Dr. Fox asked about the mitochip being developed. Dr. Barile asked whether the time from bench to bedside had improved. Dr. Tomaszewski said Taxol took about 23 years, whereas Velacade took about 8 years. Dr. Barile asked if using the new technology is safer. Dr. Tomaszewski said that it is hard to determine. Dr. Bucher asked if there are databases of human data for cancer drugs. Dr. Tomaszewski said all the data are on paper.

7. Department of Defense (DOD)

Colonel Schultheiss stated that DOD's medical research and development mission is to discover, develop, and field medical products that protect and treat warfighters serving in any environment. Examples of such medical products include the development of vaccines to bacterial or viral agents endemic to an area of operations that would otherwise deplete the fighting force, and development of a medical treatment, such as a catalytic chemical warfare agent bioscavenger, that will degrade large doses of a chemical warfare agent such as nerve agent, without self-degradation. The goal is to provide optimal medical protection and treatment for all DOD warfighters, wherever they serve.

The DOD fosters a culture of animal welfare and regulatory compliance that extends to all members of scientific, technical, and veterinary professional staffs and IACUCs who man the 36 DOD laboratories with active animal care and use programs. Each is accredited by AAALAC.

Despite an established culture to maximally use animal alternatives (the 3Rs), the nature of some of the toxins and infectious agents under investigation within the DOD requires that, in these cases, animals must be used to establish medical product efficacy. Due to the inability to perform controlled human studies when developing products against potentially deadly agents such as anthrax, the FDA relies on evidence from animal studies to provide substantial evidence of product effectiveness via the provisions of the FDA Animal Efficacy Rule, finalized in May 2002.

COL Schultheiss described the use of remote telemetry, a powerful tool with the potential for having a major reducing effect on animal use, while providing superior data collection and the potential for the use of earlier endpoints. Telemetry displays and records heart electrical activity, blood pressure, intrathoracic pressure/respiratory parameters, body temperature, and activity of animals, viewed in real time or retrospectively. Telemetry is continuous, allows for pair or group housing, and multiple sensor capabilities reduce the number of animals and experiments required. Telemetry study charts show trends over time and can possibly identify earlier endpoints to reduce pain to animals.

COL Schultheiss provided some examples of how the DOD is using alternatives. The study *Evaluation of Cytokine Activity of Three Human Tissue Constructs for In Vitro Sulfur Mustard Research* used EpiDerm, EpiAirway, and Corneal Epithelial Cell Model. The data confirmed the ability of some of tissue constructs to initiate inflammatory cytokine cascades due to mustard agent effects; however, there are some limits to these models. The U.S. Army Center for Environmental Health Research (USACEHR) developed an Environmental Sentinel Monitor to

evaluate water quality using bluegills with electrodes mounted near the gills. At the heart of the aquatic biomonitor system, an expert system integrates changes in fish behavior with data from an automated water chemistry multiprobe that tracks parameters such as temperature and dissolved oxygen that may affect fish ventilatory behavior. Changes in ventilatory behavior may indicate a toxic event. A second generation of the *in vitro* aquatic biomonitor system is evaluating cell types including immortalized frog melanocytes and primary bovine lung microvascular endothelial cells. This system is small and facilitates use in field situations. USACEHR is also doing endocrine disruptor studies using tadpoles exposed to a range of concentrations of chemicals, then allowed to develop to early adulthood. Parameters studied are sex ratios, growth, reproduction, and other developmental metrics. The frog embryo teratogenesis assay *Xenopus* (FETAX) is a 96-hour, whole-embryo developmental toxicity test to measure the effects of chemicals on mortality, malformation, and growth inhibition and is proposed as a screening assay to identify potential human teratogens and developmental toxicants. ICCVAM deemed FETAX not sufficiently validated or optimized for regulatory applications and USACEHR has no plans to further develop it. Botulinum toxin testing evaluates the serospecificity of botulinum neurotoxins to be used in pivotal animal efficacy studies and will be used to support a biologics license application to FDA. This is a possible candidate for an early endpoint or an alternative *in vitro* test. ICCVAM said the assay could be used in specific circumstances or in a tiered testing strategy; however, it could not completely replace the mouse LD_{50} test. USDA has proposed an early endpoint to botulinum studies, allowing for the euthanization of moribund animals

SACATM Comments

Dr. Fox asked if FETAX had been replaced with another assay and how it had been applied. Dr. Schultheiss said he was not sure. Dr. Fox said it was a tough assay with a lot of variability and multiple effects going on in 96 hours. Each chemical seems to affect different stages of development. Dr. Fox said they do frog work because it is hard-wired and they know almost every signal transduction pathway that is activated during development. It has a more advanced nervous system than the worm. He said there are a lot of different exposures for servicemen.

B. SACATM Discussion

Dr. Cunningham, a lead discussant, said she saw no gaps in the portfolio, but had some concern as to why some of the work, such as HTS assays, was being done and how it could help meet the objectives of the FYP. She saw a lot of overlap with HTS assays between agencies and asked if it could be more coordinated, given the limited funding. She thought the MOU is a great first step but has concerns about what will be done with the information. She stated that the projects should be thought through start to finish because the compounds are going to be more complex, e.g., nanomaterials, which have very different properties. She suggested using known compounds to optimize the screening process, before looking at the safety testing of new compounds.

Dr. Fox agreed with Dr. Cunningham and said one of the gaps is teratogenicity testing; he has not seen a lot of developmental testing. He said the agencies should be looking at developmental and fetal toxicity and suggested more interaction among the agencies to avoid duplication.

Dr. Marsman, a lead discussant, applauded the efforts and was impressed by the work being done. He suggested finding ways to communicate the ongoing work to a broader audience as an opportunity for greater stakeholder involvement and collaboration. The groups doing the work should become ambassadors for implementation of the methods into decision-making processes. He referred to Dr. Stokes' bottom-up approach with the laboratories becoming a part of the solution, thinking with "the end in mind" to make sure the assays address the decision-making process. He mentioned gaps in the FDA presentations, but only because some of the centers were not represented. He said as things like botanicals, probiotics, and herbal products are being pushed into commerce and are being scrutinized, the default should not be additional new animals tests. This integrated research program should be part of the solution to make sure that we are applying the best science and technology as we seek to protect humans and animals and environmental health.

Dr. McClellan, a lead discussant, said the presentations were excellent and he found it a very informative discussion of the relevance of the activities, but it was not clear how the different activities within agencies related to the activities at other agencies. He thought in some cases there were purposeful relationships, but with others, it was perhaps fortuitous. He said there are good interactions on a scientific level between investigators that are interested, e.g., in ocular irritation or dermal, not at the grassroots level, but more at the level of the network of agency representatives. He suggested strengthening the NICEATM-ICCVAM leadership role by clearly identifying what the portfolio is and by improving the transfer of information because there are some common themes, such as validation, that need additional information. He hoped that presentations of this type could be done on a fairly regular basis because it would be very useful to SACATM and the public. Dr. Brown agreed that establishing a portfolio would be useful and that it should include the status of the tests and any validation activities and should be disseminated outside of governmental agencies to be used by others.

Dr. Bucher responded that for the MOU, many of the things discussed in terms of the coordination of activities are really working well and could be a model for other interactions between agencies. A governance committee with Drs. Tice, Robert Kavlock, and Chris Austin is responsible for coordinating the work. There are monthly meetings between the agencies and it is really a remarkably cohesive group, moving things forward very quickly. He said regarding the redundancy, they actually see that as an advantage because there are many ways of approaching the same toxicity pathways. They don't know the best platforms to use, so they are hoping through ToxCast™ that they can sort through the platforms and use that information in other programs. He said there are some things that they can learn from the successes so far. He thanked the committee for their carefully considered and thoughtful comments.

Dr. Stokes thanked the committee and the presenters. He said when ICCVAM and NICEATM first started work on the FYP they surveyed agencies for their relevant research, development, translation, and validation activities. ICCVAM felt that it was important to invite representatives from the agencies that have the most robust R&D portfolios to make presentations to SACATM. He said the suggestions made by the SACTAM would be very helpful. ICCVAM is now establishing a R&D committee, one purpose of which is to report on the portfolio of relevant

federal activities and increase awareness across agencies. He hopes that it will be effective and that the group can serve as a nucleus to report back periodically on those portfolios.

Dr. Freeman thanked the participants, mentioned the interaction between Drs. Hamernik and McClellan, and adjourned the meeting for the day.

DAY 2 - June 19, 2008

Dr. Freeman reconvened the meeting at 8:30 and asked Dr. McClellan to read into the record the comment and question he had posed the previous day following Dr. Hamernik's presentation. He said Dr. Hamernik would have the action to provide a response from EPA.

Dr. McClellan read: "Thank you for an excellent presentation on the significant new EPA initiative. I wish to offer a comment and then follow with several questions. I am concerned that the very substantial ToxCast™ initiative is an "end-run" on the fundamental concept of the NICEATM-ICCVAM program – the use of validated alternative toxicological methods. The ToxCast™ program is using an array of *in vitro* methods that have not been validated. In my professional opinion, this raises serious questions as to whether the results can be used to predict human toxicity. One approach to correcting this serious deficiency is to make certain the ToxCast™ initiative includes a sufficiently large and diverse array of chemicals known to be human toxicants, i.e., the chemicals have well-established potential for causing human diseases, both cancer and other diseases. If this approach is used, it may be possible to validate some of the tests that are currently proposed for use in ToxCast™. In my professional judgment, the ToxCast™ approach of comparing the anticipated *in vitro* test results to the extensive toxicological data available for pesticides, largely obtained in rodents, is not a valid approach. That approach may validate the new *in vitro* tests for predicting rat or mouse toxicity; however, the real objective is to predict human toxicity and protect human health. I am encouraged that reference has been made to including 100 or so known human toxicants in the ToxCast™ program. What are these 100 or so human toxicants? How were the chemicals selected? What are the diseases they are known to cause? Will these same chemicals be included in the cooperative program being carried out at the NIH National Chemical Genomics Center?" Dr. McClellan provided a written copy to Dr. White.

Dr. Hamernik pointed out that ToxCast™ is an R&D activity and was discussed as such. She said EPA is a member of a group of other agencies that are participating in a mutual agreement, a MOU, to consider moving forward with the strategies that incorporate ToxCast™. She said she would respond to Dr. McClellan and SACATM. Dr. McClellan said he assumed that Dr. Hamernik's response would be put into the record. He said it is a very important matter and he appreciated the attention it received. [Appendix A, Dr. Hamernik's response to Dr. McClellan, is attached.]

IX. VALIDATION STATUS OF NEW VERSIONS AND APPLICATIONS OF THE MURINE LOCAL LYMPH NODE ASSAY

A. Introduction and Overview of Proposed Methods and Applications

Dr. Marilyn Wind presented the *Report on the Independent Scientific Peer Review Meeting: Validation Status of New Versions and Applications of the Murine Local Lymph Node Assay (LLNA), a Test Method for Assessing the Contact Dermatitis Potential of Chemicals and Products - Introduction and Overview*, on behalf of Dr. Joanna Matheson, Co-chair of the ICCVAM Immunotoxicity Working Group. In 2007, the timeline for the ICCVAM evaluations included the nomination from the CPSC, endorsement by ICCVAM, SACATM's endorsement of the recommended high priority for ICCVAM evaluation, and preparation of six detailed draft background review documents and draft performance standards. In 2008 the LLNA peer review panel met and a report was made available. The new/updated LLNA applications and protocols reviewed by the peer review panel included: LLNA limit dose procedure; LLNA for testing mixtures, metals, and aqueous solutions; non-radioactive LLNA: DA method; non-radioactive LLNA: BrdU-FC method; non-radioactive LLNA: BrdU-ELISA method; draft ICCVAM LLNA performance standards, and use of the LLNA for potency determinations. The documents prepared by NICEATM and the ICCVAM Immunotoxicity Working Group for each new/updated LLNA application included the draft BRD, the draft ICCVAM test method recommendations, and questions for the peer review panel.

Dr. Wind gave an overview of the murine LLNA test method protocol, explaining its initial development in 1986 by Kimber *et al.* (1986), its purpose, the dose levels used, and the stimulation index (SI). The test substance is applied to mouse ears and the mice are then injected through the tail vein with radiolabeled thymidine (or an analogue of thymidine). Lymph nodes are removed and the amount of radiolabel in the lymph node is determined as a measure of lymphocyte proliferation. A test substance with a stimulation index (SI) of 3 is considered a sensitizer.

The LLNA limit dose test method protocol differs from the traditional LLNA protocol in that only a single dose, the highest dose that does not induce systemic toxicity or excessive local irritation, is used. The LLNA limit dose test method database has data from 471 studies, representing 466 unique substances. Results with the LLNA limit dose test method almost always agree with results from the traditional LLNA. The draft ICCVAM recommendation was that the LLNA limit dose procedure should be used for the hazard identification of skin sensitizing substances if dose-response information is not needed.

Dr. Wind explained that there has been a comprehensive update of available data and information regarding the current usefulness and limitations of the LLNA for assessing the skin sensitizing potential of mixtures, metals, and substances tested in aqueous solutions. Substances used for the update included 18 mixtures, 17 metal compounds represented by 13 different metals, and 21 substances tested in aqueous solutions. Evaluating the test method performance for mixtures compared to guinea pig, the LLNA has an accuracy of 53% (8/15), a sensitivity of 50% (3/6), a specificity of 56% (5/9), a false positive rate of 44% (4/9), and a false negative rate of 50% (3/6). There were no comparative data for mixtures tested in humans.

Evaluating the test method performance for substances in aqueous solutions, the LLNA had 50% accuracy, 33% sensitivity, and 100% specificity compared to human data. Comparing guinea pig data, the false positive rate was 67%. The LLNA had 50% accuracy, sensitivity, and specificity. The false positive and false negative rates were high at 50% (n = 6).

Evaluating the test method performance for metal compounds, excluding nickel, the LLNA had 86% accuracy, 100% sensitivity, and 60% specificity compared to human data for all metal compounds (n = 14). The false positive and false negative rates were 40% and 0%, respectively. The LLNA had similar accuracy and sensitivity when compared to guinea pig data (n = 6). Based on one substance tested, the false positive rate was 100%. ICCVAM prepared draft recommendations stating that the LLNA appears useful for the testing of metal compounds, with the exception of nickel. More data are needed before a recommendation on the usefulness and limitations of the LLNA for testing mixtures and aqueous solutions will be made.

Dr. Wind reviewed the non-radiolabeled LLNA: DA test method protocol and the data from 31 substances tested by Daicel Chemical Industries. The LLNA: DA had at least 90% accuracy, sensitivity, and specificity when compared to the traditional LLNA. The draft ICCVAM-recommended use was that the LLNA: DA may be useful for identifying substances as potential skin sensitizers and non-sensitizers. The non-radiolabeled LLNA: BrdU-FC test method utilized data from 45 substances submitted by MB Research Labs. The draft ICCVAM-recommended use was that the test might be useful for identifying substances as potential skin sensitizers and non-sensitizers but more information and data are needed. The non-radiolabeled LLNA: BrdU-ELISA test method used data from 29 substances. The draft ICCVAM recommended use was that it may be useful for identifying substances as potential skin sensitizers and non-sensitizers, but more information and data are needed.

Dr. Wind reviewed the draft LLNA performance standards proposed for the assessment of versions of the LLNA that vary only from the ICCVAM-recommended LLNA by using non-radioactive vs. radioactive methods. The proposed minimum list of reference substances includes 18 substances ranging from strongly positive to strongly negative and for which there are available LLNA, guinea pig, and human data. The proposed accuracy standards are based on a chemical-by-chemical match and a set of four "optional" substances for demonstrating improved performance. She then discussed the proposed intralaboratory reproducibility standards that should be derived on four separate occasions and at least one week between tests to ensure that the tests are independent using two specified chemicals with known skin sensitizing potential.

Use of the LLNA for potency categorization as a stand-alone assay was determined using 170 substances with LLNA, human, and/or guinea pig data. The draft ICCVAM-recommended use was that the LLNA should not be considered a stand-alone test for potency categorization, but could be used in a weight-of-evidence evaluation to discriminate between strong and weak sensitizers. Dr. Wind closed her presentation with a description of the independent scientific peer panel meeting held at CPSC headquarters in March 2008 with attendance of over 50 people from five countries. The panel included experts in dermatology, toxicology, biostatistics, regulatory policy, immunology, and veterinary medicine.

B. Overview of the Panel Report

Dr. Luster presented the *Overview of the LLNA Independent Scientific Peer Review Panel Report*, starting with the charge to the panel, which was to review the draft BRDs and evaluate

the extent to which applicable validation and acceptance criteria of toxicological test methods have been appropriately addressed. Further they were to consider the ICCVAM draft test method recommendations for proposed method uses and limitations, recommended standardized protocols, test method performance standards, and proposed future studies and was asked to comment on the extent to which they are supported by the information provided in the BRD. LLNA modifications and applications evaluated included: LLNA limit dose procedure; LLNA for testing mixtures, metals, and aqueous solutions; non-radiolabeled LLNA: DA method; non-radiolabeled LLNA: BrdU-FC method; non-radiolabeled LLNA: BrdU-ELISA method; draft ICCVAM LLNA performance standards, and the use of LLNA for potency determinations.

He reported that the panel recommended the LLNA limit dose procedure, or rLLNA, which follows the traditional LLNA protocol except for the number of doses tested, for the hazard identification of skin sensitizing chemicals when dose-response information is not required. The panel also recommended that it could be used as an initial test when dose-response information is required.

The panel agreed with the ICCVAM draft recommendation for the use of the LLNA to test mixtures, metals, and substances tested in aqueous solutions and emphasized the need for the continued accrual of information (i.e., LLNA data, comparative guinea pig and human data) for mixtures, metals, and substances tested in aqueous solutions. The panel agreed with the draft ICCVAM recommendations that the LLNA: DA, LLNA: BrdU-FC, and LLNA: BrdU-ELISA non-radiolabeled test methods may be useful for identifying substances as potential skin sensitizers and non-sensitizers, but this recommendation is contingent upon receipt of additional data and information.

Regarding performance standards, the panel agreed that the use of non-radiolabeled reagents for measuring cell proliferation is a “minor” modification of the traditional LLNA protocol. Other allowable minor modifications include sex, strain, species, animals per group, and timing of test article treatment. The panel emphasized that regardless of the modification, there is the same expectation of performance and that the test method must measure only the induction phase of the immune response. They also recommended that data be collected at the level of the individual animal, that five mice per dose group be used (until reliable power calculations are conducted), and that concurrent positive controls be run until the laboratory has extensive historical data.

Regarding accuracy standards, the current database does not support the inclusion of EC3 values as a component of the accuracy evaluation. For use in hazard identification, a modified method should be evaluated with all 22 substances on the ICCVAM list (including the four optional substances) and accuracy statistics calculated. Regarding reliability standards, the panel considered using the ECt range as appropriate for the intralaboratory reproducibility analysis. They stated that the appropriateness of the 0.5x to 2.0x EC3 range for the reference substances has not been adequately justified.

The panel agreed with ICCVAM that the LLNA should not be considered a stand-alone assay for categorization of skin sensitization potency, but rather it could be used in a weight-of-evidence evaluation to discriminate between strong and weak sensitizers. More data are needed to

determine the optimal threshold in humans for distinguishing between strong and weak sensitizers.

Dr. Fox asked about the dose of BrdU and the sacrifice time following application of the chemical for the LLNA: BrdU-FC and LLNA:BrdU-ELISA test methods. He said it is important because BrdU is cytometric and expensive. Dr. Allen said NICEATM does not have a dose per weight, only a volume, which is 200 µl per mouse, and 5 hours after BrdU administration, the lymph nodes are excised for the LLNA: BrdU-FC protocol and 24 hour post injection collected for the LLNA: BrdU-ELISA. Dr. DeGeorge said the dose is administered by the weight of the animal; it is 20 µl per gram of body weight. The concentration of the BrdU injected is 100 mg/ml. He said the kinetics that were done fall between a 2 and 10-hour range, where 5 hours is the common sacrifice time. Dr. Freeman said at his company they make a standard solution and vary the volume by the weight of the mouse. Dr. DeGeorge said the information is in the BRDs.

C. Public Comments

Dr. DeGeorge registered as a public commenter and provided an annotated handout of pages 23, 24, 33, and 34 from Dr. Wind's presentation titled, *Introduction and Overview of the Proposed Methods and Applications*. He stated that although his laboratory conducts the LLNA, he is not specifically representing his lab, but is there on the basis of his experience conducting hundreds of LLNAs with various chemicals. He stated that the IP kinetics/IV dosing of BrdU can be done, though it is technically difficult, and that BrdU is less expensive than radioactive compounds. He asked SACATM to make specific recommendations that were lacking in previous expert reviews and in the tremendous amount of work that has been presented. He noted that originally the list of performance standards included 18 substances, but it was changed to add four more substances. Two tested as false positives and two as false negatives in the original LLNA vs. modified LLNA and he questioned their inclusion as test substances. Dr. DeGeorge said today was the first he had heard that 100% results would not be necessary for the modified LLNAs to be accepted. He cited the BRDs as stating that you should conduct accuracy calculations and statistics. If 18 of 18 chemicals were correct, there would be no reason in seven separate test areas to require calculations of accuracies, selectivity, and sensitivity. That number would always be 100% and anything less would fail. He believed that the true intention is not to hold the modified LLNAs to a higher standard than the original LLNA, which had an accuracy of between 72 and 86%, depending on comparisons to guinea pig or human. With respect to the flow cytometry LLNA, originally it was designed for a wide range of chemicals and included equivocal substances. In the future, picking compounds that are not clearly positive or negative should be discouraged. He stated that now the gold standard has switched. For five of the 13 sensitizers on the performance standards reference substance list, there are data from only one LLNA study for each substance.

He further stated that there would be more data for the modified LLNA than the data to which it is being compared. He called upon SACATM to espouse criteria for validation that specify a minimum accuracy and offered 90% as a reasonable number for concordance accuracy. In the case of specificity and selectivity, he suggested 80%. He considered these values to be well above the original standards and commonly recognized as acceptable. He asked SACATM to address the test method performance standards. He cited the BRDs that discuss the use of

substitutes or alternative compounds, as long as they are robust and asked SACATM to allow them. He mentioned proposed additional studies and said it should be explicitly specified whether or not they are required because the BRD says the18 chemicals need to be tested. Regarding interlaboratory reproducibility, he said you cannot move to interlaboratory validation with animals until intralaboratory validation is completed.

Kate Willett, from PETA, congratulated ICCVAM on the speed at which the review was completed. She recognized the need for development of performance standards for the methods in general, but if the comparison is between radioactive and BrdU, then the number of reference compounds is excessive. In comparing detection methods, she suggested using only a few compounds that have highly reliable data and challenging the ends of the spectrum for testing sensitivity. She then asked ICCVAM and SACATM about plans to deal with follow-up for some of the assays. She said some assays were left with no recommendation pending additional data and it sounded like additional data would be forthcoming. She asked about ICCVAM's schedule or plan for reviewing the data, because she would like to see the review completed and have ICCVAM resources spent elsewhere.

Dr. Wind responded that more data are coming in and when they get all the data ICCVAM intends to reconvene the panel to look at the new data and make recommendations.

D. SACATM Discussion

Dr. Ehrich, a lead discussant, provided written comments that Dr. White read into the record.
"• LLNA Limit Dose Procedure: 153/153 nonsensitizing agents detected and 308/318 sensitizing agents detected. The numbers make this assay look good.
• LLNA for Testing Aqueous Solutions, Metals and Mixtures: 18 mixtures tested, some without guinea pig data for validation. 17 metals tested, 12/14 sensitizers detected with 2/5 false positives. Not enough products tested to say how good this will be for metals. 21 agents at least 20% water tested but only 4 with human data, which is not enough, so can't offer opinion about this.
• Non-radioactive LLNA protocol – the LLNA DA Test Method: performance >90% for the 19 + 10 sensitizer/nonsensitizers examined, with false positives <10%. Not sure if this would be good enough for mixtures, metals or aqueous solutions.
• Non-radioactive LLNA protocol – the LLNA BrdU-FC Test Method: Flow cytometry used, with 45 test agents. Some gave equivocal results and no multi-lab studies yet. Reference studies need work. This is promising but not ready yet.
• Non-radioactive LLNA protocol – the LLNA BrdU-ELISA Test Method: This is still in progress, 23 compounds tested with an accuracy of 83%. Not detailed protocol yet. Premature to make judgments.
• Draft ICCVAM LLNA Performance Standards: no comment.
• Use of the LLNA for Potency Determinations: Purpose unclear. Was this for a validation study?"

Dr. Brown, a lead discussant, said she was a bit overwhelmed by the amount of material and focused on the final conclusions, relying on the panel and their expertise. She was impressed with the process, the number of individuals, and the thoroughness of the report. She expressed

disappointment that more conclusive recommendations could not be made from the material and that data came in too late. She asked if there were a way to make sure the data are available before setting the meeting. Dr. Brown said she shared some of the sentiments expressed by the public, such as what are the next steps. She proposed finishing this evaluation and making concrete recommendations. Tests that do not use radioactivity should get more acceptances and it is important to get the method out and get people using it. She did not find any omissions in the document. She was unclear on the purpose of the performance standards and how they would be used. She thought it should be clear what the gold standard is when asking people to provide data. The platinum standard is really what happens in humans because that is what we are trying to mimic. She said animal data are acceptable as an alternative to human data and that it is sometimes necessary to accept small sample sizes due to the limited use of alternative test methods. Dr. Stokes responded by reiterating that ICCVAM worked very swiftly once the nomination was made. NICEATM had to create the draft BRDs because the test sponsors did not submit them. He said preparing the BRDs was a huge undertaking, and test sponsors submitting complete BRDs would minimize the total review time.

Dr. Stokes said NICEATM and ICCVAM had not anticipated the difficulty in obtaining validation data and scheduled the review expecting that the data would be readily available. He said in other countries data are not provided until there is a peer-reviewed publication. This is not the case in United States and that is why there was a delay in obtaining data. He mentioned Dr. DeGeorge's comment about his data collected over the past eight years. He explained that it was a huge undertaking in terms of time and effort to obtain the original records and they did not have sufficient time or resources. Dr. Stokes said the data have been requested, some have been received, and hopefully they will get the rest. ICCVAM plans to have another expedited peer review meeting to follow up. ICCVAM is aware of the interest in these modified LLNA protocols because of the advantages offered and they are anxious to complete the review. He said agencies use an accepted traditional method in decision-making and when there is a new proposed method they always compare the performance of the new method to the existing approved method. ICCVAM is comparing new methods to both the traditional LLNA and the traditional guinea pig test because they are what the agencies accept right now. The LLNA was accepted, not because it could predict the traditional guinea pig test so well, but because its performance for predicting human sensitizers was comparable to the traditional. They will continue to assess performance of new test methods against both the currently accepted test, as well as against existing human data and/or experience, but it depends upon the data provided. He explained that they were very fortunate in getting the most robust response from industry and mentioned that the current LLNA database includes over 400 substances. compared to 200 for the original review. He acknowledged how pleased NICEATM and ICCVAM were with the willingness of industry to contribute the data, which allowed for a much more thorough evaluation of the limit test.

Dr. Charles, a lead discussant, commended the expert panel for going through the data and coming up with recommendations in the limited timeframe. He concurred regarding the inclusion of a discussion on determining the maximum dose if only a single dose is to be used in a screen process. He said you must be able to define endpoints such as "excessive irritation." He agreed with the panel for a modifying requirement that a concurrent strong positive control not be performed for every single test. The positive control is merely telling you "yes" or "no."

He asked about using a couple of animals, instead of five animals, and about doing the tests on a continuous basis. He asked how much additional work is needed to prove that the methodology is consistent and works. For the LLNA, he saw the need for the weak sensitizers, especially with regard to adding in a 1% SLS. He said, even with three animals there is pretty good correlation with the traditional LLNA, so we need further comment from the panel about the need for five animals. He concurred that four are probably needed, especially if there is adequate power in the alternative test systems. He suggested finding alternatives to the radioisotope methods. Regarding the number of chemicals used to validate the test method performance standards, five of them were ones he considered equivocal or only had one test performed on them. He suggested using chemicals with more robust data.

Dr. Dong, a lead discussant, said the panel did a wonderful job. The tables summarizing the power analysis for the modified LLNA methods are not as transparent as they should be. More footnotes or elaborations are needed for Tables 1-1, 3-1, 4-1 and 5-1 in the report. For example, the mean response and the standard deviation (SD) for the control group are not given in each of the tables, although they can be back calculated if one is familiar with the analysis procedure. He said the information is important because the SD of the response for the control group has a direct impact on the power calculations so long as the SD for the control group is assumed as the SD for the treatment group. But more importantly, the SD or variance of the control group seems to be vehicle-driven or vehicle-specific. For example, in the power calculation for the FC LLNA as shown in Table 4-1, the SD is much better when dimethylsulfoxide is used in the control group. Hence the power calculated was much higher, up to 95% with only five animals. If and when the SD or the variability of the response of the control group is vehicle-driven, then it is likely that the accuracy of the method could also be vehicle-driven. Dr. Dong said if it is too late to address this issue for the present analysis, then it should still be something that is worth considering for future studies.

Dr. Barile commended the peer review panel on a tremendous job with the amount of data submitted. He said the evaluation of the data apparently took more time than the deadline allowed. He found that some of the conclusions, statistical analysis, and the data presented from a scientific point of view rather confusing and in some incidences the conclusions were not consistent with the data. He said there were major changes throughout the study as chemicals were added in and out. If chemicals were taken out, that would alter the results of the analysis during the conduct of the studies, especially if the study were ongoing for many years. He found a bigger problem with the reference standards; 10 of the 22 chemicals were performed in only one study and he found them very difficult to compare. Another four had just two performance studies, making the majority of the reference standard done fewer than two times. He found confusing the standards used to describe accuracy, specificity, and sensitivity when comparing between the traditional LLNA and the nonradioactive methods. He also commented on the lack of the human data. He questioned the reporting of false positives in the BrdU-FC and was unclear as to the percentage being used. He questioned the use of optional chemicals and asked if they were false positives and false negatives to get a concordance with the traditional LLNA. He said ICCVAM should make sure that false positives and false negatives with the nonradioactive methods match the traditional LLNA. He questioned what constituted a 100 % concordance. He asked about the cost of the studies, and presumed it was high because of the number of animals and the labs that were asked to do these studies. He asked if it would have

been more feasible and cost-effective to wait for the additional information to come in, especially considering the time constraints on the peer review panel. He suggested giving the regional laboratories more time, reducing the number of studies, and getting clarification on the data that have been presented.

Dr. Stokes responded that there had been some confusion about the lack of data available to support the three modified LLNA protocols. ICCVAM did receive summary data for each substance for each test method, but did not receive individual animal data. ICCVAM typically requests quality assurance reports that can also be provided to the peer review panel. ICCVAM had summary data that allowed for calculation of sensitivity and specificity for each method, but not for examination of the variation among animals receiving the same dose of each chemical. With regard to selecting the 22 proposed reference chemicals for performance standards, the Immunotoxicity Working Group spent considerable time selecting the 18 chemicals and four additional optional chemicals. They started out looking at all of the 211 chemicals in the original validation database that were commercially available and applied the different criteria that are listed as to what characteristics the chemicals should have. They selected chemicals that did not produce equivocal responses and that had data using the traditional guinea pig methods as well as human data or experience. When they applied those criteria, it significantly reduced the number of chemicals from which to choose. The working group also wanted to provide a range of diversity in terms of the vehicles used and the chemical characteristics of each of the substances and sought to have a range of potency in terms of responses. So with only 13 positive chemicals and those kinds of criteria being applied, he explained that it was difficult to identify substances that had been evaluated in multiple LLNA studies, and as a result, some substances have only one study. He said ideally it would be better to have multiple studies for each substance. He reminded SACATM that these are draft ICCVAM recommendations and that after the meeting, ICCVAM will be taking the comments into consideration, along with public comments, and the report from the independent peer review panel. He said ICCVAM appreciated the comments, which will help them to revise and finalize the performance standards.

Dr. Barile said he was unsure what "level of accuracy" means. He suggested having numbers associated with accuracy, specificity, and sensitivity. Ninety percent accuracy would be considered acceptable; 80% sensitivity, specificity, also would be scientifically on target. He said it would make this summary and future summaries and evaluations much clearer.

Dr. Fox asked Dr. Luster to provide the biological basis of the assay from a molecular and cellular biology perspective. He said this is a cell-cycle reentry assay and asked whether or not the mitochondrial DNA is being measured at the same time. Dr. Luster responded that the assay is looking at the induction of the response, not the elicitation. The material is applied to the ear and the antigens are picked up by the dendritic cells in the dermis and translocated into the lymph node. If the particular T-cell recognizes a particular antigen, it undergoes cell proliferation. It is a T-lymphocyte proliferation event that eventually leads to the elicitation and the clinical response, hypersensitivity. He added that he does not think the mitochondrial DNA proliferate much and it is mostly nuclear DNA being measured in the assay.

Dr. Fox stated that he wanted to know exactly what is detected biologically and then follow up with two other questions. He said in the review for the validation, the panel recommended histopathology, but it was a weak recommendation. He said this recommendation should be considered because it is consistent or parallel with the previous recommendations for five ocular irritants. He suggested establishing histopathology if ICCVAM is going to continue with the LLNA. He thought that there must be a better alternative to the LLNA, i.e., realistically there has to be a way to assess toxicity and skin irritation better than applying a chemical to the guinea pig or mouse ears and looking at them to decide on activation. He saw no mention of any alternative to using whole animals in the report and thought it would be important to discuss an *ex vivo* or non-animal alternative. He said he calculated the dose of BrdU at 2000 µg/kg, which is a huge dose that can damage the nucleus. Dr. Stokes said the dose of BrdU is 5 mg BrdU/mouse. He said a validation study is currently being planned on an *in vitro* method for sensitization that Dr. Kojima would be talking about. ICCVAM is providing input regarding the chemicals to use for the study. Dr. Kojima said it is an *in vitro* sensitization assay being developed with ECVAM and would be ready next year. Dr. Fox asked for information on the biology of the LLNA. Dr. Luster responded that they are looking at activation of dendritic cells by looking at markers of cell division; CD1 and CD86 and several others are activated. He said the panel strongly suggested that there be some histology associated with the reduced LLNA. Dr. Stokes said they could discuss this further at the next advisory meeting.

Dr. McClellan questioned the change in time period and suggested some simpler approaches to comparing BrdU to tritiated thymidine. Dr. Tice responded that in every test method evaluation ICCVAM does, they look at how reliable the method is and how accurate or relevant it is in predicting the particular event that is used for classification. With the reduced LLNA, the question was: does it perform as well as the traditional method given that you are only using one dose level rather than three? In the case of the three alternative methods, each method was compared independently against the original radioactive LLNA. Even taking into account the small changes in protocol, one of the issues to address is whether those changes were considered to be minor changes or major changes, where a major change might have an impact on the performance of the assay. In the ICCVAM guidelines on the LLNA, the OECD test guidelines, and the EPA guidelines, it specifies the use of male CBA mice. Another strain of mouse or another sex of CBA can be used if you demonstrate that it doesn't impact the performance of the assay. Performance is assessed through accuracy and reliability. Performance standards were not available at the time that the original LLNA was evaluated. Performance standards are used to help accelerate the validation of an alternative test method that is functionally and mechanistically similar to an existing test method. Had those performance standards existed, they would have been used, both in the development and evaluation of the non-radioactive methods. Considering that performance standards didn't exist then, ICCVAM is not holding those assays to those standards, but they are looking to see how they perform in that context. The working group also looked at expanding the applicability domain because the traditional LLNA is not considered useful for metals. There weren’t enough data on complex mixtures and on aqueous solutions. The use of LLNA for metals was a re-evaluation compared to the radioactive methods, which might have impacted also on the nonradioactive methods. Dr. Tice explained that the panel had to work through a fairly complicated scenario. NICEATM tried to set up the test methods for the panel in sequential fashion to prepare them for what they evaluated later during the meeting.

Dr. Wind said she wanted to make sure that everyone understood that ICCVAM knew the methods being developed were nonradioactive test methods. One of the reasons the LLNA wasn't being used more widely is that there are a number of countries where the use of radioactivity is not allowed, and, in addition, there are difficulties associated with using radioactivity. She said ICCVAM thought it was important to look at nonradioactive LLNA methods; however, they did not develop those methods. She said the methods were under development and were brought to the Immunotoxicity Working Group for review. She noted that performance standards make it easier for "me too" assays to be developed and not have to go through the same rigorous validation process as the original assay. She said the Europeans were pushing for the assay to be used as a "stand-alone." It is possible with the LLNA to make a determination of up to five different potency categories. CPSC staff felt that this was very important, particularly since under the GHS, there was an expert group examining the use of LLNA in determining classification based on potency categories. She explained that the panel addressed numerous questions, which is why is the review seems so confusing.

Dr. McClellan expressed concern that such a complex structure has been created for validating new tests. He said it will result in only a few new tests being available in 10 years and suggested occasionally stepping back from the rules.

Dr. Freeman said the discussion illuminated the issue of the roles that ICCVAM, NICEATM, the committee, and the agencies play in terms of promulgating the tests in a way that can impact our society in a regulatory fashion. Dr. McClellan agreed and said he thought this meeting had been one of the best because of the breadth of the agenda and opportunities for SACATM to provide advice.

Dr. Stokes appreciated SACATM's insights and precautionary concerns. ICCVAM has advocated, from the very beginning, communicating and interacting with assay developers. When this occurs, ICCVAM connects them with regulatory scientists who have experience in that particular toxicity endpoint to discuss validation study designs and protocols before they conduct a validation study. This interaction enables ICCVAM to work with them on the appropriate design of the study and selection of the appropriate chemicals that should be used to generate the data needed by regulatory agencies to make decisions on whether that test is acceptable for the purpose that it is proposed for. He said if you look at the number of chemicals and the number of laboratories that have been used for the data for these three methods, if the performance standards had been available for the developers to use, significantly fewer number of animals would have been used at a lot less expense. Laboratories have generated probably three times as much data as ICCVAM has proposed in the draft performance standards. He said this is ICCVAM's attempt to try to get ahead of that curve and get the performance standards out there for use by test method developers. ICCVAM routinely provides performance standards now with every new method. If performance standards had been developed in 1998, it would have benefited and expedited the development and validation of these three non-radioactive LLNA methods.

Dr. Fox concurred with Dr. McClellan in not understanding the 24-hour BrdU vs. the 5-hour BrdU. He said the half-life of BrdU is only 2 hours. He suggested ICCVAM use a different

approach in regarding assay reviews, such as bringing the proposed assay to SACATM to get input on whether it's an appropriate assay to review or if the appropriate questions are being asked in its review. Dr. Stokes said the suggestion seemed reasonable as a way to proceed in the future, whenever possible.

X. REPORT ON THE SCIENTIFIC WORKSHOP ON ACUTE CHEMICAL SAFETY TESTING: ADVANCING *IN VITRO* APPROACHES AND HUMANE ENDPOINTS FOR SYSTEMIC TOXICITY EVALUATIONS

A. Introduction and Objectives

Dr. Stokes introduced the background and objectives of The Scientific Workshop on Acute Chemical Safety Testing: Advancing *In Vitro* Approaches and Humane Endpoints for Systemic Toxicity Evaluations held on February 6 – 7, 2008 in Bethesda, MD. The workshop was organized and sponsored by ICCVAM-NICEATM, ECVAM, and JaCVAM. Safety testing for acute systemic toxicity to determine the poisoning potential of products is important for public health because more than 4 million poisonings occur annually in the United States and poisonings are the second leading cause of injury-related deaths. Safety testing provides the basis for accurate hazard labeling, risk management, and informed treatment decisions. The 2000 ICCVAM International Workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity reviewed the validation status of *in vitro* approaches for acute systemic toxicity and recommended strategies and research needs to further reduce and eventually replace animal tests with *in vitro* methods.

He outlined the current strategy for refining and reducing animal use in acute oral toxicity testing. Study directors must look at all the information about a chemical and consider if it is appropriate to use an *in vitro* cytotoxicity test to help establish a starting dose. Animal studies can be refined to reduce pain and distress by applying earlier endpoints; however, it will always be impossible to accurately model acute systemic toxicity in *in vitro* test systems if there is little or no understanding of the mechanisms causing the animal to exhibit acute adverse clinical signs or of the causes of death. Accordingly, the goal of this workshop was to identify how to obtain mechanistic information from *in vivo* testing conducted currently to meet regulatory requirements to help inform the development of sufficiently accurately *in vitro* models that might eventually replace animal use. The workshop also sought to identify objective clinical signs and biomarkers that might serve as earlier, more humane endpoints to reduce and preferably avoid pain and distress in such studies. The long-term goal is replacement of animals with an integrated battery of *in vitro* methods that predict acute systemic toxicity using human cells and tissues.

Dr. Stokes acknowledged ongoing R&D activities in Europe such as the A-Cute-Tox Project, which has the aim of developing a simple and robust *in vitro* testing strategy that could predict human acute systemic toxicity potential accurately and potentially replace animal toxicity tests currently used for regulatory purposes. This project implements the R&D recommendations

from the 2000 International Workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity.[1]

Alternative methods for acute oral toxicity testing developed through ICCVAM include the Revised Up-and-Down procedure in 2001, which reduces animal use by 70%. Dr. Stokes said the rationale for the workshop, alternatives for acute systemic toxicity testing, is one of ICCVAM's four highest priorities; it is a goal of the FYP. Also, the EU is seeking non-animal approaches to meet the ban on using animals for acute systemic safety testing of cosmetic ingredients in March 2009. He mentioned that the goals of the workshop are consistent with the NRC report, *Toxicity Testing in the 21st Century*, and NTP's *Vision for the 21st Century*. The workshop could contribute to those visions by developing predictive pathway based *in vitro* methods for acute systemic toxicity testing as a proof-of-concept and by discussing approaches to identify the key toxicity pathways for acute systemic toxicity. Better understanding of these pathways could also lead to identifying biomarkers that could be used as earlier, more humane endpoints in *in vivo* testing.

Dr. Stokes said the workshop, organized by NICEATM, ICCVAM, ECVAM, and JaCVAM had over 120 attendees from six countries. Workshop goals were to: (1) review the state-of-the-science and identify knowledge gaps regarding key *in vivo* pathways involved in acute systemic toxicity; (2) recommend how these knowledge gaps can be addressed by collecting mechanistic biomarker data during currently required *in vivo* safety testing; (3) recommend how *in vivo* key pathway information can be used to develop more predictive mechanism-based *in vitro* test systems and identify biomarkers that may allow earlier, more humane endpoints; and (4) recommend how mechanism-based *in vitro* test systems and earlier more humane endpoints can be used to advance the 3Rs while ensuring the continued protection of human and animal health. The workshop was divided into four sessions: (1) Acute Systemic Toxicity: Public Health Significance and Regulatory Testing Needs, (2) Acute Systemic Toxicity: Human and Animal Assessment, Biomarkers, and Key Pathways, (3) Humane Endpoints, and (4) State of the Science: Using *In Vitro* Methods to Predict Acute Systemic Toxicity.

The workshop report will be published in 2008 and the workshop summary will be published in *Environmental Health Perspectives*. Dr. Stokes closed by acknowledging the invited workshop participants, the ICCVAM Acute Toxicity Working Group, ICCVAM agency representatives, and NICEATM staff that were involved with this project.

B. Workshop Recommendations

Dr. Wind reported on the five breakout groups' objectives, conclusions, and recommendations.

Breakout group 1: Key Pathways for Acute Systemic Toxicity had the objectives of discussing the current understanding of the key pathways for *in vivo* acute systemic toxicity; identifying and prioritizing future research initiatives; and reviewing molecular, cellular, tissue, physiological, and clinical biomarkers that are or could be measured or observed during *in vivo* acute systemic

[1] ICCVAM. 2001. Report of the International Workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity. NIH Publication No. 01-4499. Research Triangle Park, NC: National Institute for Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/.

toxicity testing. The group (1) recommended key pathways that should be further studied to better understand the toxic effects of chemicals and to better understand and treat acute human poisonings; (2) identified knowledge gaps related to the diagnosis and treatment of human poisonings; (3) identified toxicological observations and measurements to address these gaps; and (4) recommended research and development activities.

Breakout group 2: Current Acute Systemic Toxicity Injury and Toxicity Assessments had the objective of discussing and identifying observations and quantitative, objective measurements that could or should be included in current *in vivo* acute systemic toxicity tests to elucidate key toxicity pathways that would support the future development and validation of predictive *in vitro* methods. The group provided recommendations (1) for biomarkers expected to provide more information and a better understanding of the pathophysiological effects and modes/mechanisms of acute systemic toxicity in current animal tests and (2) for activities to obtain more information on key toxicity pathways from the current *in vivo* acute systemic toxicity tests.

Breakout group 3: Identifying Earlier Humane Endpoints for Acute Systemic Toxicity Testing had the objective of discussing what *in vivo* data collected to elucidate key toxicity pathways might lead to the identification and validation of earlier, more humane endpoints for acute systemic toxicity testing, and what data should be a priority for collection to aid in identifying earlier, more humane endpoints. The group (1) reported on the use of biomarkers to identify earlier humane endpoints for acute toxicity tests; (2) reported on research, development, and validation activities relative to humane endpoints; and (3) discussed implementation of recommended activities relative to humane endpoints.

Breakout Group 4: Application of *In Vivo* Mode of Action and Mechanistic Information to the Development and Validation of *In Vitro* Methods for Assessing Acute Systemic Toxicity had the objectives of (1) discussing how key toxicity pathways indicated by *in vivo* measurements (molecular, cellular, tissue, or other physiological, and clinical biomarkers) and observations are currently modeled or could be modeled using alternative *in vitro* test methods and (2) identifying and prioritizing research, development, and validation activities for *in vitro* test methods that model the key *in vivo* toxicity pathways and more accurately predict acute systemic toxicity hazard categories. The group provided recommendations regarding (1) *in vivo* toxicity pathways to be modeled by *in vitro* systems (e.g., integrated batteries of test methods, high throughput test methods, genomic and other –omics approaches); (2) *in vitro* modeling of *in vivo* acute systemic toxicity; (3) knowledge gaps related to *in vitro* modeling of *in vivo* acute systemic toxicity; (4) research, development, and validation activities; and (5) implementation of these recommended activities.

Breakout Group 5: Industry Involvement in Test Method Development, Validation, and Use had the objective of discussing how to promote the collection and submission of *in vitro* and *in vivo* toxicity test data to ICCVAM to advance the development and validation of more predictive *in vitro* test methods and earlier, more humane endpoints for acute systemic toxicity testing. The group provided recommendations regarding the current uses of *in vitro* cytoxicity testing by industry and submission of *in vitro* and *in vivo* data to ICCVAM.

C. Public Comments

Kate Willett, PETA, noted that some of the sessions at the meeting were quite productive, but that the charge questions and the people participating were not the right mix to actually progress the level of discussion very far. She said some of the breakout groups discussed the same things, i.e., the potential biological pathways involved in acute toxicity. She felt there was a lack of expertise in this area among the people involved and that many of the key pathways have already been identified. She thought the discussion could have started at a higher level if some of the experts from around the world had been invited to the workshop (especially participants in the A-Cute-Tox Project) and that the breakout groups could have been organized around the *in vitro* technology already in progress.

D. SACATM Discussion

Dr. White read into the record the written comments of Dr. Becker, a lead discussant: "Question 1: see discussion of Breakout Group 5 of the Acute Safety Testing workshop; research to develop cost-effective techniques to enable such measurements (reality is that methods must be cost effective… if they are not, then this will be a major barrier to their use); consider having a central lab (government funded?) to generate the data (clinical path, histopath, etc.) and serve as a repository for this data and the *in vivo* data; need a defined set of procedures to collect meaningful data for method validation, which suggests a centralized approach (it won't help if disparate methods are used which have markedly different variabilities, etc.); if results could be generated with little or no cost or transaction effort, then this would be more likely to be successful; public–private consortium to facilitate data collection and submission - See discussion of Breakout Group 5 of the Acute Safety Testing workshop." Question 2: "Recommended biomarkers/measures that are sufficiently predictive and ready to use now (from the Acute Safety Testing workshop in February, 2008): behavioral observations (already conducted, cage side observations and detailed clinical observations) – most labs have SOPs for these. If this is to be a formal FOB this will be costly and therefore not likely to move forward; body temperature changes; body weight and feed and water (sometimes included already). What would be possible means to have such data generated by industry? The reality is that industry testing of this type is largely dictated by regulations. Therefore, when such regulations incorporate these endpoints into the test guidelines and protocols required by regulatory agencies, data will be generated.

As far as other recommendations go for measurements and observations that are not currently done routinely: there is a considerable need for research to develop cost-effective techniques for obtaining such measurements. The reality is that these methods must be cost effective… if they are not, then this will be a major barrier to their use; consider having a central lab (government funded?) to generate the data (clinical path, histopathology, etc.) and serve as a repository for this data and the *in vivo* data. Need a defined set of procedures to collect meaningful data for method validation, which suggests a centralized approach (it won't help if disparate methods are used which have markedly different CVs/variations etc.). *Ad hoc* validation efforts generally are not very useful. Different labs can use different protocols, etc., and this limits or can even completely prevent data use for a validation review (example: ICCVAM peer review of the relatively straight forward ER binding assay). If results could be generated with little or no cost or transaction effort, then this would be more likely to be successful. For

biochemical/histopathology endpoints: Study is conducted in Lab X per usual procedure. Samples are taken and shipped to the Central Lab, and Central Lab conducts the new measurements. Study Lab reports the results of the usual procedure to Central Lab and Central Lab these results up with their results of the new endpoints.

Question 3: Consider specific funding for research to standardize and validate these methods. Right now there are considerable funding sources in the US for basic research (method development) but identifying sources for funding for validation (standardization and actual validation studies) is difficult. Per the report of the SACATM Working Group for the NICEATM-ICCVAM Five-Year Plan: It would be very beneficial for NICEATM/ICCVAM to include more details, at least for the select set of highest priorities that speak to the specific elements of research and development, translation, and validation (available at http://ntp.niehs.nih.gov/files/SACATMFYPWGReport061207.pdf).

From Page 5: The focus for the current federal funding seems to be for basic research; therefore, new, revised, and alternative methods will continue to have a very difficult time making it from the researcher's bench into a regulatory testing regimen. Formal validation is a necessary step that must be achieved for a test method to be adopted and used in a regulatory program–so this raises the question, "Are there gaps that exist in the planning (or lack of planning) for these validation activities?" If so, the Five-Year Plan should identify these gaps. Furthermore, even if translation and validation studies were given greater attention, without a strategic plan in place, NICEATM and ICCVAM agencies will not have a clear path forward to devote to focusing these activities on the highest priority methods or areas."

Dr. Barile, a lead discussant, discussed some strengths of the report, which include the incorporation of mechanism of action, targeted organ toxicity, in-cell systems, alternatives, and the coupling of that information with the volumes on basal cytotoxicity that has been accumulated over the years. He said the development of *in vitro* tests for prediction and delineation of toxic mechanisms, not just for screening, is a step forward and can provide information that can be used to mimic human toxicity. He did not think it is going to be helpful for either animal or *in vitro* tests to be able to predict diagnostic measures in humans, as those are predominately reported by observational and clinical case studies. Alternative methods could be of great value in the treatment, follow-up, and understanding of the toxicity e.g., mimicking one hour or up to 24-hour alternative toxic tests and understanding reversibility and recovery in the cell test and the cumulative effects. Cell tests have the advantage of time and two-year studies are not needed. Cumulative tests can be reproducible using population doublings within the culture system, allowing these cells to double over a week's time, which can be equivalent to generations of cells. Dr. Barile stated that the cumulative nonspecific binding to macromolecules could be measured.

He said a weakness of the report is breakout group 4, the industry response to developing acute systemic toxicity tests. He referred to slide 25 that the current cost-benefit ratio does not justify validated *in vitro* methods to set starting doses. The number of animals being used is at a minimum and is dependent upon the species. Use of higher species (dogs and primates) has been kept at the same level or gone down, but the number of rodents has not gone down. He sees it as an impediment to implementing alternative methods and a completely misguided response. Dr.

Barile did not recommend forming a consortium to facilitate data collection and submission. He understood the regulations and constraints that industry is put under for product development; however, progress has to move forward toward developing alternative tests. Dr. Barile suggested moving forward with trying to develop tests that target the mechanisms as well as screening tests. The federal agencies and Congress should know that funding for test development is inadequate. He said implementing these activities will be difficult and that funding must be made available for development, research, and training. He said the field would not progress if industry is allowed to just be concerned with the bottom-line figures.

Dr. Diggs, a lead discussant, was pleased to be a part of the workshop and thought it was very productive. She thought identifying earlier endpoints in acute systemic toxicity evaluation would have a significant impact on the reduction of animal use in research and minimize pain and distress, ultimately. She said ICCVAM needs to identify the specific mechanistic data that they are looking for and even request identified data from specific industries or institutions. She suggested doing additional work in identifying biomarkers, both those already available and being collected and those that need to be identified. Those biomarkers should then be communicated to the agencies and industry for implementation. The use of earlier endpoints might actually save time and money. She said ICCVAM must identify the data gaps, clarify them, and then communicate them back to the agencies and stakeholders.

Dr. DeGeorge, a lead discussant, said ICCVAM should identify areas of industry (product and service companies, non-profit organizations) that might be easily approachable. He suggested encouraging sponsors to code and submit data, which may be feasible if confidentiality can be guaranteed. He suggested including in the NIH Guide for the Care and Use of Laboratory Animals ("The Guide") some guidelines on additional things to incorporate into studies and information to collect such as blood, serum, tissues, and cell lysates to look for microRNA and mitochondrial DNA. ICCVAM could be a central repository for such collections. Dr. DeGeorge suggested doing retrospective studies with the coded blind tissue, media, and lysates. He said creating confidentiality agreements with product and technology companies to protect them after submission of non-validated R&D mechanistic data would make then more likely to submit information. To fill data gaps, he suggested reaching out to the lab animal science community (e.g., AALAS) and the veterinary community and to provide training for veterinarians in toxicology.

Dr. Freeman commented that with regard to reaching out to industry, some of the discussion the previous day might be relevant, such as how outreach was done in the ocular studies. He said there is a balance in industry labs, the science side that generates the data and the animal welfare side. He encouraged reaching out to IACUCs. In addition to having a means to ensure confidentiality of data submissions, he said another factor is that industries may have requirements under TSCA or other legislation regarding data. If data are generated and considered important, there is a responsibility to report it; however, a company might interpret that responsibility more conservatively than the regulators. Questions also arise about how data are reported on material safety data sheets. He said it is not just about repercussions coming back from regulators or protecting proprietary information, but more far reaching and somewhat insidious.

Dr. McClellan said he was not impressed with the make-up of the workshop panel. He said there are thousands of different mechanisms of acute intoxication and death related to the range of different agents, which must be recognized at the onset. It is an oversimplification to think there will be a magic solution and there are many misstatements of fact regarding different sectors of industry. He said this issue is not a real high priority and it is important to emphasize the rationality that needs to be brought to the table when doing acute toxicity studies. In some cases, the likelihood of an exposure occurring that could result in acute intoxication is so remote that is not worth expending one animal.

Dr. Brown said she had misinformed Dr. Barile about the number of animals used in research, and that only a small percentage of them are used in toxicology, but many are used in transgenic research. She said people should have access to funding for validating alternative methods. She concurred that data submissions to agencies needs to be coded and they should be looked at as predictive, not necessarily for a specific chemical, but for a class of chemicals. Companies should be able to submit data without future repercussions. She said there is currently an effort to revise The NIH Guide, and suggested that people attend meetings and give input on the 3Rs. Regarding weighing animals at every clinical observation, she said not all test animals are rodents, and it is not easy to weigh non-human primates without stress and risk of injury to both people and animals. She said a spectrum of species is used in toxicology, so they should not make rules about weighing without thinking more broadly. Dr. Barile said he understood that the increase in the number of rodents was not just in the toxicology field, and that there are increases in pharmacology and biological sciences as well.

XI. NOMINATION TO ICCVAM: NTP RODENT BIOASSAY FOR CARCINOGENICITY

A. Presentation

Dr. Raymond Tice, Deputy Director, NICEATM, discussed the ICCVAM nomination to evaluate the validation status of the NTP rodent bioassay to accurately predict human carcinogens and noncarcinogens. The nomination was received on October 24, 2007, through the NTP on-line nomination process anonymously. ICCVAM is asked to evaluate the suitability of results obtained using this test method as a standard against which the predictive performance of alternative test methods can be measured objectively, and, if the test method cannot be validated, what new data/approaches would be needed to conduct a proper evaluation. On January 23, 2008, ICCVAM considered the nomination in conjunction with currently available information on the test method's usefulness and limitations, and proposed that its evaluation be assigned a "low priority." On April 23, 2008, ICCVAM reviewed and agreed to the "Draft ICCVAM-Recommended Priority" document for the NTP rodent bioassay and on May 7, 2008, the NTP published a Federal Register notice announcing the SACATM meeting, listing the nomination as an agenda item, and requesting public comment.

Dr. Tice explained that SACATM would consider the draft ICCVAM priority for the nomination and later this summer, ICCVAM would consider all inputs and materials on the nomination including public comments and SACATM comments in setting its final evaluation priority. The draft priority is based on an evaluation of the ability of the assay to accurately identify human

carcinogens and noncarcinogens. He said it represents the proposed current priority for evaluating the performance of this test method; however, ICCVAM and NICEATM recognize that future planning and priorities must be flexible in order to take advantage of opportunities resulting from advances in science and technology and development of new test methods and to respond to new testing needs. Dr. Tice then posed the question for SACATM: Do you agree with ICCVAM's draft priority for this nomination? If not, please explain.

B. SACATM Discussion

Dr. Freeman asked Dr. Stokes for clarification on why SACATM was being asked to vote, and when is a vote required or not required. Dr. Stokes responded that in 2003, ICCVAM published a process by which anyone can make nominations to ICCVAM for any type of activity related to their mandates. It could be to nominate a new method that they think may be promising or a test method to undergo validation studies. Once nominations are received, NICEATM provides background information and refers it to an appropriate ICCVAM working group, if one has been established for this specific toxicity area, to develop a draft priority and draft a recommended activity. In this case, ICCVAM considered the nomination and voted unanimously to give it a draft low priority and not proceed further with the nominated activity. The next step in the process is to solicit public comment and bring the draft priority and draft recommended activity to SACATM for comment and their views on what priority the nomination should have and what activity they think is most appropriate. Dr. Stokes explained that SACATM is charged with discussing what priority they think it should have and then voting on that. Typically nominations are assigned high, moderate, or low priority and comments are provided on the recommended activity.

Dr. Freeman asked how many existing test methods (ocular, dermal, or cancer bioassays) have been reviewed before by ICCVAM and how this nomination fits into the purview. Dr. Stokes responded that normally, when ICCVAM gets a new alternative test method for review, they look at it for its usefulness for predicting human health effects, if that's the purpose of the test. ICCVAM also reviews the predictivity of the traditional existing assay to the extent that human data are available to make such an assessment. He said in this case, there is not a new alternative method related to carcinogenicity being presented to ICCVAM to evaluate, so there isn't the need at this time to make this assessment. That factored into the draft priority the ICCVAM committee decided to apply to this. If they were considering a new alternative method for carcinogenicity, then ICCVAM would have to evaluate any existing test method data and to compare the relevance and the reliability of the proposed new test method versus that of the existing test method.

Dr. Dong, a lead discussant, stated that both sides did not provide enough information or detail regarding the nomination. For example, ICCVAM states that based on the priorities described in the FYP, any further evaluation of this assay should have a low priority at this time. However, unless ICCVAM has a comparison analysis done on these priorities, there are no grounds for their statement. He said he would not give the nomination a high priority, but a moderate priority would likely not move the nomination forward. He felt that evaluating the current protocol for the two-year bioassay would be a reasonable exercise to determine if modifications are needed, but realized that ICCVAM may have other priorities for the next five years.

Dr. Freeman mentioned that Dr. Frank Johnson, NIEHS, provided written comments on the nomination that were provided to SACATM members.

Dr. Marsman, a lead discussant, said he respected and appreciated the nomination. The rodent bioassay is a research-directed test that originated with NCI, before the days of NTP. It was used in an R&D context to understand the carcinogenicity of agents and only later turned into more of a regulatory tool for understanding exposure to humans and the risk of these agents. He was not sure it ever had the kind of validation towards humans that it maybe could have or should have. With that caveat, he concurred with the placement of the carcinogenicity assay in the FYP, putting it in the second tier. He said other endpoints are of more immediate concern, some of which will begin to address the complexity of developing non-animal alternative methods for systemic toxicity. Ideally, all of the *in vivo* test methods should be validated against human data and understood in the light of the human response. He said the rodent bioassay might be one of the more difficult methods to validate, but that it could be addressed in the context of known human carcinogens. Much of what the assay is used for is to evaluate carcinogenicity for compounds for which human data are lacking. We have a wealth of information on the background incidences of cancer types in humans, but no direct causal evidence linking them to a particular agent at low doses, so a full validation of the cancer bioassay would be limited.

Dr. McClellan, a lead discussant, looked at the nomination with considerable interest, having conducted a number of bioassays predating the NTP, and was involved in establishing the core protocol and modifications that extended the observation period to two and half years. He noted that over time the design has been altered to include parallel studies of toxicokinetic data and ancillary studies that gave insights into the mechanism of action, but that it has been difficult to get these other studies incorporated into the NTP protocol. He stated further that he was delighted yesterday during the NTP presentation to see how things are emerging in terms of alternative approaches. Extensive work done on the NTP database shows the bluntness of the tool, and the extent to which a carcinogenic outcome can be predicted from knowledge of the effects in terms of the MTD. He commented that the bioassay yields a large number of false positives; however, it is important to recognize that the results of these studies have a wide impact and play a major role in the NTP's biennial Report on Carcinogens and at IARC and other government agencies. He said a lot of emphasis is given to what is basically a yes or no answer as to whether or not a substance induces cancer. It gives a misleading impression to other scientists and the public about toxicants and that it is critical to move beyond a yes or no answer to understand the potency of the material. Dr. McClellan said if the nomination is given a low priority at the present time, then it should be done so recognizing that the rodent bioassay is not a validated method. This is important in terms of any future actions that come to SACATM to evaluate an *in vitro* test related to carcinogenicity or other endpoints that come out of these bioassays. It is important to compare the *in vitro* results to the human data and not start with the rodent bioassay as the gold standard because may be a flawed *in vivo* assay. He expressed enthusiasm for a critical review of the NTP 2-year rodent bioassay using the rigorous validation methodology of NICEATM-ICCVAM, but thought it would be inappropriate to proceed now with the validation because the resources are not at hand to do the *extensive* review that is needed.

Dr. Barile stated that he was pretty much in agreement with the general comments of the rest of the group, given the limited resources that are available to ICCVAM. ICCVAM has done a monumental job at promoting alternative methods with their available budget. Also, in consideration of the ICCVAM mission to advance alternative methods, setting the NTP rodent assay at anything but a low priority would place a considerable strain on ICCVAM's resources.

Dr. Freeman asked for a vote on the nomination. SACATM voted 8 yes, 0 no to concur with ICCVAM's recommended low priority for the nomination.

XII. PROPOSAL FOR INTERNATIONAL COOPERATION ON ALTERNATIVE TEST METHODS

A. Presentation

Dr. Stokes presented the proposal for the International Cooperation of Alternative Testing Methods (ICATM), which was developed by ICCVAM, ECVAM, JaCVAM, and a representative from Health Canada in response to a request from the International Cooperation on Cosmetics Regulations (ICCR). ICCR is a voluntary international group comprised of the U.S. FDA, Health Canada, the European Commission Directorate General Enterprise, and the Japanese Ministry of Health, Labour and Welfare. The ICCR's purpose is to provide a multilateral framework to promote free trade by identifying ways to remove regulatory obstacles among the regions, while maintaining the highest level of global consumer protection. The first meeting was on September 26-28, 2007, at which they discussed alternatives to animal testing and prepared a statement that the ICCR recognizes the importance of the 3Rs, that it welcomes the efforts of industry and validation centers in this effort, and that collaborations and communications in the design, execution, and peer review of validation studies should be further strengthened. The ICCR invited ICCVAM, ECVAM, JaCVAM, and a representative of the Government of Canada to address this issue, and to propose options to ensure a collaborative approach.

Dr. Stokes noted that there are currently numerous collaborations among ICCVAM-NICEATM, ECVAM, and JaCVAM. However, these current collaboration are on an *ad hoc* informal basis, and the level of coordination and communication varies widely for any given test method. While these collaborations are highly beneficial to all parties, such efforts require additional resources in terms of time and funding. The validation organizations also have very different processes for evaluating the validation status of test methods. The lack of consistent coordination, as well as different processes, have led to differences in the recommendations among the organizations on the usefulness and limitations of alternative methods for regulatory purposes.

The initial ICATM concept was developed over a series of teleconferences and meetings in the spring of 2008. The proposed goal of ICATM is to achieve international cooperation necessary to ensure that new alternative test methods adopted for regulatory use will provide for equivalent or improved protection of people, animals, and the environment while reducing, refining, and replacing animal use wherever scientifically feasible. ICATM's purpose is to promote international cooperation, collaboration, and communication among national validation organizations in order to ensure optimal design and conduct of validation studies, ensure high

quality independent scientific peer reviews, enhance likelihood of harmonized recommendations by national validation organizations, avoid duplication of effort, and leverage limited resources to achieve greater efficiency and effectiveness.

The proposed initial ICATM membership is NICEATM-ICCVAM, ECVAM, JaCVAM, and Heath Canada, with the future inclusion of other members decided by consensus of the members. Dr. Stokes explained that ICATM provides a framework for enhanced international cooperation, collaboration, and communication in three related, but independent, critical stages: test method validation studies, independent peer review of the validation status of test methods, and development of formal test method recommendations for regulatory acceptance consideration. The heads of each member organization will be responsible for ensuring cooperation, communication, and coordination by their respective organization. All decisions will be by consensus and will respect the national laws, policies, rules, regulations, and directives of the member organizations.

Dr. Stokes further articulated that for validation studies, there should be information-sharing prior to the validation effort with the objective of developing consensus on critical aspects of validation studies before the study starts, including the validation study design, proposed test method protocols, proposed reference chemicals, and proposed regulatory purpose. For independent scientific peer review, draft test method recommendations and background review documents provided to the panel should be publicly available, and the report of the peer review panel should be made publicly available. The objective is to conduct peer reviews in a manner that will meet the needs of all validation organizations and thereby avoid the need to repeat peer reviews in each country. In the development of final test method recommendations, the goal is for each of the validation organizations to develop harmonized ICATM recommendations that are then forwarded to respective national regulatory authorities and international test guideline organizations. The ICATM member organizations are responsible for ensuring consistent coordination, cooperation, and communication; providing opportunities for stakeholder involvement; and committing time and resources to optimize the processes. Success will be indicated by consensus among ICATM members on the usefulness and limitations of new alternative methods and more rapid national and international acceptance of alternative methods.

B. SACATM Comments

Dr. Cunningham asked about the role of ICCR in the proposed ICATM and about the status of agreement with the other three members of the ICATM. Dr. Stokes responded that the organizations are still in the discussion phase, but have agreed on the major areas to be addressed. Standardizing the different independent peer review processes is something they need to work on, so they do not have to be repeated in different countries. Regarding the ICCR, the ICATM proposal is being developed further by an ICCR working group in response to a charge from the ICCR. The revised proposal will go to the ICCR for their review and comment. While the ICATM will work independently of ICCR in the future, it will also be available to address issues the ICCR might refer them to. Dr. Bucher emphasized that creating an international agreement of this type is not an insignificant endeavor from the standpoint of getting permission at the various levels in our own government, let alone other entities. It is a proposal at this stage, for comment on the concept and the elements of it; it is not a done deal.

Dr. Stokes explained that in the United States, the State Department has the responsibility for all international agreements. So anything of this nature will have to be done consistent with State Department regulations and requirements.

Dr. Diggs asked about the relationship between ICCR and this group. Dr. Stokes responded that the ICCR requested that the validation organizations work together to develop a proposal for increased cooperation and the ICATM proposal that he presented is their response. The ICCR working group, which includes representatives of the three validation organizations and Health Canada, will forward the proposal to the ICCR for comment. Dr. Diggs asked about funding mechanisms for the group and whether decisions are binding. Dr. Stokes said each of the four organizations would be responsible for obtaining the funds to support an adequate level of participation. He added that the ICATM would not make decisions that are binding, just as ICCVAM recommendations are not binding on any organization. ICATM will simply serve as a framework to facilitate harmonized recommendations among the four centers. There will not be ICATM recommendations on test methods, rather recommendations by each organization that will ideally be the same. Dr. Diggs asked about the peer review process being sponsored at the ICATM level. Dr. Stokes said one organization will have the lead for a specific test method's independent scientific review, but would involve the other organizations including requesting nominations of experts that could serve on the review panel. When this is led by an organization other than ICCVAM and NICEATM, it will occur outside of this country.

Dr. McClellan asked for a summarization of the reviews conducted by the four entities in the last six years. Dr. Stokes said Canada has not undertaken any reviews, except through receipt of peer review results from the OECD and the other international organizations. Dr. Blakey of Health Canada said Canadian scientists have served on several peer review panels organized by ICCVAM and ECVAM. Dr. Stokes said previously there have not been publicly available peer review reports, except from the United States, although ESAC recently agreed to make their future peer review reports publicly available. He said public availability of peer review reports and background review documents will help speed the international consideration and adoption of new alternative methods. For example, after ICCVAM evaluated the LLNA and the Up-and-Down Procedure, it provided the peer review reports and background review documents to OECD with the proposed test guideline. These alternative test methods were subsequently considered and adopted by the OECD in an unusually short period of time. When such thorough documentation has not been available, the OECD review and adoption process has taken four or more years. Dr. Kojima said Japan has had five or six peer reviews. Dr. Linge said Europe has had 34 methods over the past 17 years for which the ECVAM scientific advisory committee ESAC has issued validation statements. Twelve of those were replacement methods, sixteen were refinement and reduction, and eight were for general purposes. Dr. McClellan said he would like to see ICATM go forward, while maintaining the independence of each entity. Each country has responsibilities to look after and the United States has some differences that must be resolved. Dr. Stokes said 17 alternative methods have been adopted since 1999 in the United States. Ten of those are based on technical evaluations that included detailed BRDs and peer review through the ICCVAM process. He said he understood that 11 of the 34 methods in Europe have been adopted by regulatory authorities and four others are now included in the European Pharmacopoeia. Some of the other 19 methods do not have regulatory applicability and some have not been accepted.

Generally, where there has been a thorough evaluation of all related issues for a test method, including public independent scientific peer review, regulatory approval has been expedited. Dr. Stokes gave as an example the disagreement that occurred with the usefulness of *in vitro* skin corrosivity tests. There was agreement on the performance of the test with regard to sensitivity, specificity, false positives, and false negatives. However ECVAM and its ESAC viewed the tests as valid replacements for the rabbit test because ECVAM considered them to be more predictive than the rabbit test. This conclusion was based on expert opinion that the rabbit tests had at least a 20% false positive rate and at least a 20% false negative rate for dermal corrosivity; however, this assertion was not substantiated by scientific data. ICCVAM did not agree that the methods could be considered valid replacements based on the public health implications of the known false negative rates. ICCVAM recommended that the tests could be used in a tiered testing strategy, where positive results could be classified as dermal corrosives, but chemicals testing negative would require further testing in animals to identify corrosive substances that were negative in the *in vitro* test. An analysis of the actual test data by ICCVAM concluded that there was a 5% possibility of a false negative for substances that were borderline in the weak corrosive/strong irritant category, and a 0% possibility for moderate and strong corrosives. With regard to false positives, ICCVAM concluded that there was no biologically plausible way that non-corrosive substances could cause false positive results in the rabbit corrosivity test. Dr. Stokes said if there had been closer collaborations between the organizations at that time, there could have been a more thorough discussion of this issue during the peer review and evaluation process and likely could have avoided such a significant difference in recommendations on the test method usefulness and limitations. Dr. Diggs said she was concerned that this coordination would bog some processes down, just the opposite of what they are hoping for. Dr. Stokes acknowledged that international coordination may require more time to complete a review, but would result in a greater likelihood of harmonized recommendations.

Dr. McClellan asked about the role of the State Department. Dr. Stokes said development of the cooperation proposal is being done under the auspices of the ICCR international agreement, for which the FDA is the designated U.S. member. He noted that FDA was holding a public meeting on the same date as the SACATM meeting where they were accepting public comments on the ICCR activities. Dr. Brown said this initiative appears to be driven by the cosmetics industry and asked about other industry involvement and the possibility of them concurring with this arrangement. Dr. Stokes said FDA has had two public meetings, giving opportunity for written and oral public comment by any interested organization or industry. He reiterated that the ICATM is a framework to facilitate and assist the involved validation organizations with each developing similar recommendations on the scientific validity of new, revised, and alterative test methods; it is not an organization with authority. It is formalizing a process to accomplish what some of the organizations have been doing for the past 10 years and allowing them to do it on a consistent basis.

Dr. Brown asked about setting priorities, about other models for this type of collaboration, and about ICCVAM recommendations for moving forward. Dr. Stokes answered that ICCVAM and NICEATM are asking SACATM for comments on the ICATM proposal. He mentioned that the ICCR framework seems to be working well, and the ICATM proposal is in fact modeled to a large extent on the ICCR agreement. Regarding ICATM priorities, he said they would probably

not go to SACATM; rather ICCVAM would seek advice from SACATM on priorities, and use this advice in setting the priorities that they felt should be addressed by the ICATM organizations. Dr. Bucher explained that this activity is called for in the FYP to promote harmonized adoption of methods. Dr. Cunningham asked about the role of SACATM in moving forward. Dr. Stokes said there are currently representatives from three of the four ICATM member organizations who are non-voting *ad hoc* members of the SACATM. In addition to the 15 ICCVAM agency principal representatives, this also includes representatives from ECVAM and JaCVAM. Accordingly, they are all made aware of SACATM views and advice. In addition, he and Dr. Wind have observer status on the ECVAM scientific advisory committees, which helps ensure a timely exchange of information and awareness of activities at ECVAM. Updates on ICATM activities and progress would of course be presented to SACATM at its meetings. Dr. Bucher said the functions and authorizations of SACATM come from the ICCVAM Authorization Act of 2000 and those functions would not change. Dr. Stokes quoted text from the Act that authorizes efforts relevant to international harmonization: "ICCVAM shall . . . facilitate appropriate interagency and international harmonization of acute or chronic toxicological test protocols that encourage the reduction, refinement, or replacement of animal test methods."

C. Public Comments

Dr. Hamernik asked about individual ICCVAM-member agencies' concerns and possible disagreements with recommendations of ICATM, or the representatives to ICATM, and the regulatory needs of U.S. member countries. She said the structure presented seemed to be one level removed from individual ICCVAM-member agency input and representation. She asked about potential conflicts regarding individuals from ICCVAM-NICEATM, who are also interacting with ICCVAM-member agencies, to possibly get them to accept recommendations from ICATM for the sake of harmonization. Dr. Stokes responded that the goal is harmonized recommendations from each ICATM validation organization that would then be forwarded by each validation organization to their respective national regulatory authorities, and, as appropriate, international test guideline organizations. He said the wording on one slide may be misleading because, in fact, ICATM will not make test recommendations; rather, they will be made by each of the validation organizations. The goal is to have recommendations issued from all four entities that are harmonized. He emphasized that the ultimate decision on ICCVAM recommendations lies with ICCVAM, and this would not change. Regarding conflicts with statutory authority of member agencies, he said ICCVAM recommendations have no status as regulatory requirements; the law states that each regulatory agency considers ICCVAM recommendations and makes acceptance decisions based on their regulatory needs and statutory responsibilities. Dr. Hamernik asked about minority opinions from ICCVAM-member agencies. Dr. Stokes said in accordance with the current processes, any minority views on ICCVAM test method recommendations would be recorded and forwarded with the recommendations. However, in the first 10 years of ICCVAM, the committee has always been able to reach consensus on all test method recommendations so there have been no minority opinions.

Sara Amundson, HSLF and HSUS, said she wanted to provide some public policy historical perspective regarding the interest of the U.S. State Department. The number one trade consideration of the EU, as expressed by their Trans-Atlantic Economic Cooperation Council,

which is a subset of a sort of State Department consideration both in the EU and the United States, ensures that we have consensus around the EU Cosmetics Directive because of the impact on U.S. industry. That began this dialogue, which stretched out more specifically to the implementation of the cosmetics directive and harmonization here in the United States to ensure that our U.S. trade was not going to be negatively impacted. It will also stretch more proactively to address those harmonization considerations under REACH. She said this is important because the U.S. government recognizes that there is not currently a process in place outside of the OECD to really ensure that this harmonized activity takes place at the very onset of consideration of specific methods. From that perspective, the animal protection community is supportive, over all, of what is being considered. She said her one consideration has more to do with the EU and both the Cosmetics Directive and REACH. They have deadlines that they have to meet. She said utilizing this sort of opportunity for harmonization they might not place themselves in a situation where they are bogged down in meeting their legal set of deadlines. She asked Drs. Stokes and Linge to address this issue. Dr. Stokes acknowledged the sense of urgency in Europe with the impending deadline for the Cosmetics Directive. He said Dr. Thomas Hartung provided timelines for best-case scenarios which project that they will not be able to meet the March 20009 deadline for ocular and acute oral toxicity. The science, mechanism-based predictive methods, and associated validation studies are not available yet. ICCVAM is trying to help by expediting development and adoption of the BCOP and ICE *in vitro* ocular test methods as international test guidelines rather than guidance documents. The ICCVAM Ocular Toxicity Working Group and NICEATM plan to forward the proposed test guidelines to OECD in July. Dr. Linge agreed that the deadlines are tight and that they want to speed up validation and regulatory acceptance. He mentioned speeding up the process by doing just one peer review. In complex biological systems, two peer review panels may come to slightly different conclusions and recommendations. By harmonizing the peer review process, it may overcome the "not-invented-here" problem. But too many committees and stakeholders actually slow down the process. He said ECVAM cannot afford to slow down the process, but want to speed it up.

Dr. McFarland asked if ICATM would be limited to cosmetics and not to other parts of FDA's purview. Dr. Stokes answered that ICCR requested a framework and though their interest is in test methods for cosmetics, in reality, the test methods used for cosmetics are the same test methods used for other product sectors. While the framework is being developed under an ICCR umbrella and request, it will be a freestanding agreement among the four validation organizations that would apply to all of their work on test methods. Since there are no test methods limited specifically to cosmetics, it would be impossible to limit the cooperation to cosmetics. Nonetheless, priorities and progress in alternative methods are being driven by that product sector now. Dr. McFarland asked about a specific tie-in to ICH at this point. Dr. Stokes said that any ICCVAM recommendation coming forward could be taken by the US FDA, the U.S. lead organization for the ICH, to the ICH for consideration, or similarly by the Center for Radiologic Devices and Heath, the U.S. lead organization for the International Standards Organization. ICCVAM recommendations are made available to any regulatory agency to move forward for consideration by applicable international organizations.

D. SACATM Discussion

Dr. Cunningham, a lead discussant, said she agreed with the concept and thought it is a great step forward, but she had some concerns regarding obtaining consensus on all of the decisions ICATM will be making. She expressed concern about how member organizations will be added and how they can maintain their voting and decision ability with increased membership. A third concern was how ICATM would facilitate consensus in the peer review process to have one guideline. She asked about having a combined scientific advisory board to ICATM. Dr. Stokes said there was an agreement by ESAC at its May 2008 meeting to make the reports of their peer review panels publicly available, which has never been done before. This is a significant positive development, and should greatly assist other validation organizations with their evaluations of the same method. He said the ESAC discussed the need for transparency and opportunity for stakeholder involvement. He reiterated that ICATM is a framework to facilitate discussion and exchange of information, and is not an autonomous unit making independent decisions. He said an independent international scientific advisory board would be redundant because each of the existing organizations has its own advisory committees and liaisons, which works very well. He said international agreements are not easy and have a lot of challenges and barriers to overcome, but he considers that the benefits are well worth the efforts that it takes to develop and implement enhanced cooperation.

Dr. Diggs, a lead discussant, said ICATM was a good concept and hoped that ICCVAM-NICEATM and the other validation organizations will move forward with it. She acknowledged that it would be a huge task requiring tremendous effort.

Dr. Marsman, a lead discussant, applauded the approach. He said the ICATM framework would promote much better free trade by identifying ways to remove regulatory obstacles among the regions and maintain a high level of protection of consumer, animal, and environmental health. In addressing the issues of exposure in the products that his company develops, they interact with a variety of regulatory agencies in the United States. Multiplying that by the >170 countries in which they market products, it becomes a daunting task to begin to understand all of the complexity of trying to get countries to understand the development of new assays. He said anything to lower the barriers to those communications pathways would be much welcomed. Anything that would streamline or improve the consistency of the processes, the expectations, the reciprocity, the acceptance, and the implementation of all those kinds of methods are the kinds of things that he would actively support. He discussed differences in the statements of purpose between ICCR and ICATM. He said it is really important to set a framework for the strategy for future work and to address the fact that there is a diversity of stakeholders that use an assay, not just registrants and regulators. He supported the value Dr. Stokes placed on a bottom-up approach to hazard identification, getting feedback from stakeholders in the strategy setting stage to have the opportunity to make a hard and fast definition of what the objectives and goals are for the development of the new assay. They can then set a strategy for execution against those goals for new methods and would be in a position to clearly define what the measures of success are for the methods.

Dr. Fox said he applauded the idea and is looking even more to the future, seeing it as a possible venue to get countries like China and India, both with good toxicologists, to be influenced by four leading countries to promulgate alternative methods. This body, with a harmonized

viewpoint, would carry more weight than just one country. He sees this in a futuristic mode having a positive impact on global alternative animal use.

XIII. UPDATES ON ECVAM AND JaCVAM

A. Update on JaCVAM

Dr. Hajime Kojima, Director of JaCVAM, presented a brief overview of ongoing validation studies and peer reviews. *In vivo* comet validation studies are underway and *in vitro* studies will start in August. The LUMI-CELL¸ Estrogen Receptor Assay international validation, lead by NICEATM, will have phase IIa studies completed this June. The peer review of the Stably Transfected Transcriptional Activation (STTA) assay determined that it could be used for estrogen agonist testing.

New validations studies in 2008 include the estrogen antagonist STTA assay using 15 chemicals, the Cell Transformation Assay using Bhras cells, the cytotoxicity test of Short Time Exposure (STE) Eye Irritation assay using 25 chemicals, the EPISKIN method using 19 chemicals, and the h-CLAT/THP-1 cell skin sensitization assay using ~40 chemicals.

Dr. Kojima then updated SACATM on independent peer review and recommendations to regulatory agencies. Workgroups on immunotoxicity, ocular toxicity, dermal corrosives, and acute toxicity will be started shortly. He described a battery system to predict phototoxicity using yeast growth inhibition and red blood cell photohemolysis assays. Shadow peer review meeting on skin irritation (EPISKIN) and ocular irritation (BCOP and ICE) are in progress and skin sensitization, pyrogen screening, rLLNA, and acute systemic toxicity will be started soon.

Regulatory acceptance is in progress for (1) alternatives to animal testing for safety evaluation of the cosmetic ingredients and quasi-drugs, (2) 3-D human skin models for skin corrosivity, and (3) LLNA-DA. Dr. Kojima explained the category classification in various countries of cosmetics, drugs, and quasi-drugs, which includes those used as colorants, disinfectants, and for sunscreens, hair growth, breast rash, and acne.

A regulatory acceptance board was established in 2007 to review alternatives to animal testing for safety evaluation of cosmetic ingredients and quasi-drugs. Seven task forces were established: skin irritation, skin sensitization, skin penetration/absorption, eye irritation, phototoxicity, genotoxicity, and acute toxicity.

Dr. Kojima stated that JaCVAM hopes to work with ICCR and ICATM. Included in the collaboration will be the Japanese Cosmetic Industry Association, the Japanese Society for Alternative Animal Experiments, and the JaCVAM steering committee, regulatory acceptance board, and advisory board.

B. Update on ECVAM

Dr. Jens Linge gave an overview of EU actions on the 3Rs that include the Cosmetics Directive and its amendments; REACH regulation; Directive 86/609/ECC (protection of animals for

laboratory use); ECVAM's activities within the Joint Research Centre since 1991; EU-funded R&D projects totaling $140 million in the past five years; the Community Action Plan on protection and welfare of animals; and the European Partnership for Alternative Approaches to animal testing (EPAA). He described the steps from R&D, pre-validation, validation, peer review, and EU regulatory acceptance to OECD regulatory acceptance. He mentioned the 7th Amendment of the Cosmetics Directive, which created an urgent need for alternative methods, especially for complex toxicity endpoints, due to the phasing out of ingredient testing with test and marketing bans in 2009 and 2013. Toxicological endpoints for which ECVAM considers that methods are ready before these dates are: (1) skin corrosion, (2) phototoxicity, (3) skin irritation, (4) eye irritation for severe irritants, (5) skin absorption/penetration, (6) photogenotoxicity, (7) acute toxicity, (8) skin sensitization, and (9) genotoxicity and mutagenicity (micronucleus and COMET test). Toxicological endpoints for which methods are not ready are: (1) subacute and subchronic toxicity and repeated-dose toxicity, (2) toxicokinetics and metabolism, (3) carcinogenicity, and (4) reproductive and developmental toxicity, embryotoxicity, and strategies from the ReProTect project.

Skin irritation is a key area of topical toxicity. The Skin Irritation Validation Study and Test Chemical Selection have been published. Peer reviews are currently underway for the SkinEthic assay and the validation study on the optimized EpiDerm assay. Eye irritation is also a key area. A retrospective validation of cytotoxicity-/cell function-based assays is planned. Other efforts include human reconstituted tissue models (e.g., EpiOcular) and organotypic assays. In the key area of sensitization, efforts include workshop reports, *Evaluation of Chemical Reactivity Methodologies for Screening Skin Sensitisation Potential*, and *An Evaluation of Performance Standards and Non-Radioactive Endpoints for the LLNA*. A manuscript, *Progress in the Development of New Approached to the Identification of Respiratory Allergens*, is in preparation. Prevalidation/validation studies are forthcoming for peptide binding assays, dendritic cell-based assays, and vitosens. Efforts are also directed toward the LLNA (reduced LLNA, performance standards, and a non RI-LLNA).

Dr. Linge discussed efforts in the key area of genotoxicity (the MNT *in vitro* validation study, the COMETassay, the 3D-skin model and false positives in genotoxicity testing) and carcinogenicity (prevalidation of the cell transformation assays). He described ongoing studies, workshops/publications, and international collaborations in the area of kinetics. New strategic developments include robotics platforms, "omics" and profiling, and developmental neurotoxicity testing. New efforts in biologics and food include two workshops on vaccines, a report, *Overview of the Test Requirements in the Area of Food and Feed Safety*, and a validation study for the Toxiline-DSP test. In ecotoxicology, new work includes a bioaccumulation study of *in vitro* trout, an acute aquatic toxicity meeting, and a HESI subcommittee on animal alternative needs in environmental risk assessment.

New endocrine disruptor work includes optimization of ReProTect; validation of the PANVERA-ER binding test, HeLa ER antagonist, and LUMICELL ER agonist and antagonist; and regulatory acceptance of the OECD test guideline of the HeLa agonist ER validation study. He discussed the workshops and publications on "Triggering and Waiving Criteria for the Extended One-Generation Reproduction Toxicity Study," and explained that reproductive toxicity testing uses 60% of all animals. Progress has been made on the alternatives database

(DB-ALM), with 1151 registrations from 64 countries and downloads of over 300 documents/month. Dr. Linge provided a timeline for ECVAM LLNA performance standards and provided a comparison of the European perspective with that of ICCVAM in terms of positive controls, number of animals per dose group, and individual vs. pooled lymph node cells.

Dr. Hamernik asked about the scientific questions being asked with regard to the carcinogenicity assays. Dr. Linge said they wanted to label the substance as carcinogenic or noncarcinogenic and to distinguish between genotoxic and nongenotoxic substances.

XIV. OTHER BUSINESS

Dr. Freeman brought up scheduling of the next meeting and asked if meeting annually is appropriate. He asked about the topics and if there were gaps. Dr. Brown said they should not meet more often, just for the sake of meeting, but if an important topic necessitates meeting before a year, then it should be done. Dr. Stokes said NICEATM and ICCVAM have taken that into consideration, and will request more frequent meetings if there are action items that need to be addressed in a timely manner, such as commenting on peer review panel reports. Dr. Marsman thanked Dr. Stokes for extending the meeting to two days, which allowed for more thorough discussion than in the past. He agreed with Dr. Brown that they should meet on the basis of need. He suggested possible phone conferences for things like commenting on the second LLNA peer review panel report, which would allow SACATM to provide feedback on an interim basis without physically getting together. Dr. Brown suggested a webinar format, but acknowledged the limitations of the public meeting requirements. Dr. Charles concurred about meeting earlier and using the two-day format. Dr. Stokes thanked the SACATM members and said this meeting had been most helpful in terms of feedback and advice for ICCVAM and NICEATM. He also thanked the ICCVAM agency representatives for their participation. Dr. Bucher and Dr. Freeman thanked everyone. Dr. Freeman adjourned the meeting at 4:45 PM.

Appendix A

Answer to Dr. Roger McClellan from Presentation of Dr. Karen Hamernik at the June 18-19, 2008 SACATM Meeting

Thank you for your questions regarding the U.S. EPA's ToxCast™ research program. The three-phased ToxCast™ program (see www.epa.gov/ncct/toxcast and Dix et al., (2007), Toxicol.Sci. **95** (1):5-12) is being developed to provide a biological basis for the prioritization of chemicals for toxicological testing and hence, is aligned with the challenges set by the National Academy of Sciences report on Toxicity Testing in the 21st Century. Achieving this goal will help provide important information needed for the toxicity assessment of thousands of high and medium production volume chemicals, pesticidal inerts and other environmental contaminants of concern to the EPA. ToxCast™ is not intended as a replacement for any current toxicity assay. The objective of Phase I (Proof of Concept) of the ToxCast™ program is to use more than 18 assay sources to complete the biological activity profiles (e.g., derivation of chemical signatures) of more than 300 chemicals (mostly pesticides) whose toxicity has been well characterized using standardized test guidelines for assessing developmental, reproductive, chronic, and subchronic endpoints. All the information generated in the ToxCast™ program will be made available to the public for independent evaluation. ToxCast™ is a major contribution of the EPA to the Tox21 Memorandum of Understanding (MOU) between the EPA and federal partners at the National Toxicology Program of the NIEHS and the NIH Chemical Genomics Center (NCGC) at the National Human Genome Research Institute. The Governance Board of the MOU is comprised of the Director of the NCGC, the Director of EPA's National Center for Computational Toxicology, and the Chief of NTP's Biomolecular Screening Branch. As such, there is close coordination between the efforts of NIH and EPA on the program.

Phase II of ToxCast™ is intended to validate (confirm) the predictivity of any biological signatures derived in Phase I by examining another group of up to 300 additional well characterized chemicals (Phase IIa). Recognizing the ultimate desire is to predict human toxicity, and not rodent toxicity, plans for Phase IIb of ToxCast™ include the examination of approximately100 additional chemicals for which robust data on toxicity in humans are available. The 100 human toxicants are yet to be selected. Ideally these chemicals will have both preclinical and clinical data on toxicity in animals and humans, respectively, and will be drawn from the universe of pharmaceutical agents that have not advanced to the later stages of the drug safety evaluation process. EPA has entered into discussions with the ILSI-HESI DART (International Life Sciences Institute, Health and Environmental Sciences Institute, Developmental and Reproductive Toxicology Technical Committee) to develop a plan by which the candidate drugs for Phase IIb could be provided by participating pharmaceutical companies. Since the chemicals in ToxCast™ are a subset of those being screened at the NCGC as part of the Tox21 Initiative, the Phase IIb drugs would be part of the larger screening library. These drugs are anticipated to be identified by late 2008, but this will depend on successful efforts of the ILSI-HESI working group. As with other parts of the ToxCast™ program, Phase II will be conducted in as transparent a manner as possible. If the research program is successful, Phase III will provide application of the approach to those chemicals of concern to the various program offices in EPA so that they can be prioritized for traditional animal testing. You and other interested parties are encouraged to participate in the monthly teleconferences of the Chemical Prioritization Community of Practice as a way to remain up to date on the program (see http://www.epa.gov/ncct/practice_community/).