Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM)

June 17 – 18, 2010

Minutes from the June 17 – 18, 2010 SACATM Meeting

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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 (*http://ntp.niehs.nih.gov/go/7441).*

3Rs	Replacement, reduction, and refinement (causing less pain and distress) in the use of animals for toxicological testing
AWA	Animal Welfare Act
BrdU	bromodeoxyuridine
BCOP	Bovine Corneal Opacity and Permeability
CDER	Center for Drug Evaluation and Research
CFR	Code of Federal Regulation
CM	Cytosensor Microphysiometer®
CPSC	Consumer Product Safety Commission
CRO	contract research organization
CVB	Center for Veterinary Biologics
DACLAM	Diplomate, American College of Laboratory Animal Medicine
DA	Daicel Adenosine Triphosphate
DMSO	dimethyl sulfoxide
DOD	Department of Defense
ECVAM	European Centre for the Validation of Alternative Methods
ED	endocrine disruptor
ELISA	enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
ER	estrogen receptor
ESAC	ECVAM Science Advisory Committee
EU	European Union
FDA	Food and Drug Administration
FYP	NICEATM-ICCVAM Five-Year Plan
GHS	Globally Harmonized System
HHS	Health and Human Services
IACUC	Institutional Animal Care and Use Committee
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.
IPCS	International Programme on Chemical Safety

IRE	Isolated Rabbit Eye
JaCVAM	Japanese Center for the Validation of Alternative Methods
KoCVAM	Korean Center for the Validation of Alternative Methods
KFDA	Korean Food and Drug Administration
KSAAE	Korean Society for Alternative to Animal Experiments
LLNA	Local Lymph Node Assay
MAQC	MicroArray Quality Control
MOC	Memorandum of Cooperation
MRI	magnetic resonance imaging
NC	National Coordinators (OECD)
NCTR	National Center for Toxicological Research
NIAID	National Institute of Allergy and Infectious Diseases
NICEATM	NTP Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences
NIFDS	National Institute of Food and Drug Safety
NIOSH	National Institute for Occupational Safety and Health
NIH	National Institutes of Health
NRC	National Research Council
NTP	National Toxicology Program
OECD	Organisation for Economic Cooperation and Development
PHS	Public Health Service
QSAR	Quantitative Structure-Activity Relationships
QSDAR	Quantitative Spectra Data-Activity Relationships
SACATM	Scientific Advisory Committee on Alternative Toxicological Methods
SOT	Society of Toxicology
TSCA	Toxic Substances Control Act
USDA	U.S. Department of Agriculture
WG	working group

II. Attendance

SACATM met on June 17 – 18, 2010, at the U.S. Environmental Protection Agency, 109 T.W. Alexander Drive, Research Triangle Park, NC 27711. The following individuals attended the meeting:

SACATM

James Freeman, PhD, ExxonMobil Biomedical Sciences, Inc., *Chair*
Laura Andrews, PhD, DABT, Genzyme Corporation
Karen Brown, PhD, Pair O'Docs Enterprises
George Corcoran, PhD, ATS, Wayne State University
Helen Diggs, DVM, DACLAM, Oregon State University
Marion Ehrich, PhD, VA-MD Regional College of Veterinary Medicine
Eugene Elmore, PhD, University of California, Irvine
Steven R. Hansen, DVM, MS, MBA, DABT, ABVT, American Society for the Prevention of Cruelty to Animals

Gwendolyn McCormick, DVM, MS, DACLAM, Boehringer Ingelheim
Sharon A. Meyer, PhD, University of Louisiana at Monroe
Steven Niemi, DVM, Massachusetts General Hospital
Michael Olson, PhD, ATS, GlaxoSmithKline
Annie (Peiyong) Qu, PhD, University of Illinois-Champaign
Linda Toth, DVM, PhD, Southern Illinois University School of Medicine
Gary Wnorowski, MBA, LAT, Eurofins/Product Safety Laboratories

Liaison Representatives
Joachim Kreysa, PhD, ECVAM
David Blakey, PhD, Health Canada (by telephone)
Soon Young Han, PhD, KoCVAM

ICCVAM Primary Representatives
Jack Fowle, III, PhD, DABT, EPA
Jodie Kulpa-Eddy, DVM, USDA, *ICCVAM Vice-Chair*
Paul Nicolaysen, VMD, NIOSH
RADM William Stokes, DVM, DACLAM, NIEHS, *NICEATM Director*
Margaret Snyder, PhD, NIH
Kristina Hatlelid, PhD, CPSC

Other ICCVAM Representatives
Raj Chhabra, PhD, DABT, NIEHS
Richard McFarland, MD, PhD, FDA/Center for Biologics Evaluation and Research
Donna Mendrick, PhD, FDA/National Center for Toxicologic Research

Invited Speakers
Alicia Karas, DVM, Tufts Cummings School of Veterinary Medicine
Joseph Haseman, PhD, Independent Consultant

***Ad Hoc* Discussants**
Alan Proia, MD, PhD, Duke University
Robert Peiffer, MD, Merck

NIEHS/NIH Staff
Terry Blankenship, DVM
Linda Birnbaum, PhD, DABT, ATS
John Bucher, PhD, DABT
Warren Casey, PhD, DABT, *NICEATM Deputy Director*
Sally Fields
Robbin Guy
Jean Harry, PhD
Robin Mackar
Debbie McCarley
Raymond Tice, PhD
Mary Wolfe, PhD
Lori White, PhD, PMP (*Designated Federal Officer)*

Image Associates Staff
John Maruca
Steven McCaw

SRA International Staff
Brent McCuen

Breakthrough Staff
Ernie Hood

ILS Staff (NICEATM support contractor)
David Allen, PhD
Tom Burns, MS
Frank Deal, MS
Jonathan Hamm, PhD
Nelson Johnson
Brett Jones, PhD
Elizabeth Lipscomb, PhD
Linda Litchfield
Steven Morefield, MD
Anna Lee Mosley
Michael Paris
Eleni Salicru, PhD
Catherine Sprankle
Frank Stack
Judy Strickland, PhD, DABT
Linda Wilson

Public

Samantha Suiter, People for the Ethical Treatment of Animals
Karen Barlet
Cliff Broadway
John Gordon, PhD, Alion Science and Technology
Patrick Hayden, PhD, MatTek Corporation
Chae-Hyung Lim, DVM, KoCVAM
Shulei Zhao, PhD, CertiChem, Inc.
Sue Leary, Alternatives Research & Development Foundation
David Kurtz, DVM, Experimental Pathology Laboratories, Inc./US-EPA

June 17, 2010
III. Welcome and Introductions

Dr. James Freeman, SACATM chair, called the meeting to order at 8:30 A.M. Individuals in the room introduced themselves. Dr. Linda Birnbaum, NIEHS and NTP Director, welcomed everyone to the meeting, thanked SACATM members for their dedicated service, and noted the knowledge and perspective they bring to Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). She further acknowledged the ICCVAM agency representatives and the NICEATM staff for their ongoing commitment and dedicated efforts. She noted the contributions of Dr. Marilyn Wind, Chair of ICCVAM from the Consumer Product Safety Commission (CPSC), and Dr. Jodie Kulpa-Eddy, Vice-Chair of ICCVAM from the U.S. Department of Agriculture (USDA), for their leadership. She welcomed the international partners, Dr. Joachim Kreysa, European Centre for the Validation of Alternative Methods (ECVAM), Dr. Soon Young Han, Director of the newly established Korean Center for the Validation of Alternative Methods (KoCVAM), Dr. David Blakey, Health Canada, (joining by teleconference), and Dr. Hajime Kojima, Japanese Center for the Validation of Alternative Methods (JaCVAM), who was unable to attend. She noted the Memorandum of Cooperation (MOC) for International Cooperation on Alternative Test Methods (ICATM) and KoCVAM's participation in joint validation studies with NICEATM and JaCVAM.

Dr. Birnbaum emphasized the vital role that NICEATM and ICCVAM serve in protecting, promoting, and advancing the health and safety of our citizens. The NIEHS mission is to discover how the environment causes or contributes to injury and disease, and to use this knowledge to reduce and prevent injuries and disease that can result from such exposures. NICEATM and ICCVAM serve a key role in translating these research advances and new technologies into scientifically valid safety testing methods for regulatory use. Improved injury and disease prevention requires effective translation of new knowledge into better test methods, or "public health prevention tools." Test methods that accurately detect whether chemicals and products can cause injury or disease are vital to prevention. New test methods are expected to not only be more predictive, but also faster, cheaper, and to require fewer or no animals.

Protection of workers and consumers demands that new alternative methods continue to accurately identify chemical hazards so that they have appropriate warning labels. Accidental and improper exposures to chemicals have a potentially significant public health impact. New test methods must continue to accurately detect whether chemicals and products can cause injury or disease, so that we avoid under-labeling that could contribute to even higher rates of chemical injuries.

ICCVAM and NICEATM continue to provide an extremely effective process for achieving the regulatory acceptance of new safety testing methods. ICCVAM has now contributed to the endorsement or adoption of 33 alternative methods. Eighteen of these are *in vitro* methods, half of which use human cells. Thanks to ICCVAM's continued focused efforts, there are now approved alternative tests for many different types of tests, including five of the six most commonly conducted safety tests. Most

recently, Federal agencies have accepted ICCVAM recommendations for updated procedures for assessing allergic contact dermatitis (ACD) that can reduce animal use by up to 50% compared to the original test approved ten years ago. In addition, since the last meeting, the international regulatory community, through the

Organisation for Economic Cooperation and Development (OECD), has adopted the first *in vitro* methods that can be used worldwide to identify some chemicals that may cause blindness or severe eye injuries.

Last week Dr. Birnbaum forwarded ICCVAM test recommendations to Federal agencies that are the first to incorporate "green technology." These are two versions of the Local Lymph Node Assay (LLNA) that incorporate new biomarkers that eliminate the use of radioisotopes, thus benefiting animal welfare as well as the environment. She also forwarded ICCVAM recommendations to the Health and Human Services (HHS) Secretary on alternative methods and approaches for eye safety testing. ICCVAM has recommended the routine use of analgesics, anesthetics, and humane endpoints whenever animals must be used for eye safety testing. Adoption and use of these procedures is expected to eliminate nearly all discomfort previously involved with this important test. She congratulated ICCVAM and NICEATM on their many accomplishments this year, which have laid the groundwork for greater progress to be more rapidly achieved in the future. The use of new alternative methods has and will continue to have a huge positive impact on animal welfare. ICCVAM's careful evaluations will ensure that these methods continue to support and improve the protection of people, animals, and our environment. Dr. Birnbaum closed by presenting certificates and letters of appreciation to the four members of SACATM who completed their terms, Drs. Helen Diggs, Marion Ehrich, James Freeman, and Annie Qu. She also announced that a Society of Toxicology (SOT) Stem Cell Specialty Section is being started and needs charter members. Dr. Michael Waalkes of NIEHS is the contact person.

Dr. John Bucher, NTP Associate Director, welcomed the attendees and thanked them for participating. He said the mission of ICCVAM and NICEATM is very important to the NTP and to animal welfare around the globe. He thanked Dr. Freeman for chairing SACATM and Dr. William Stokes for leading NICEATM. Dr. Kulpa-Eddy, ICCVAM vice-chair, thanked the members of SACATM on behalf of ICCVAM and looked forward to receiving advice. Dr. Lori White, Designated Federal Officer, read the conflict of interest statement for SACATM.

IV. NICEATM-ICCVAM Update

A. Presentation

Dr. Stokes, NICEATM Director and ICCVAM Executive Director, welcomed everyone on behalf of ICCVAM and NICEATM and thanked SACATM members for their participation on the advisory committee. He acknowledged the continued participation and hard work of ICCVAM members and the scientists who have served on working groups (WGs). Dr. Stokes provided updates on NICEATM-ICCVAM activities.

• *Alternative Methods for ACD Safety Assessments*: ICCVAM completed final reports and recommendations, which were forwarded to Federal agencies. They also submitted two new test guidelines (TGs) and an updated TG 429 to OECD. NICEATM and the Immunotoxicity WG are continuing to contribute to the ECVAM-led validation studies on two new peptide reactivity assays and two *in vitro* assays for ACD via liaison members on the validation management team. ICCVAM evaluations and final recommendations were completed for the (1) updated LLNA protocol; (2) reduced LLNA protocol (rLLNA); (3) LLNA performance standards; (4) two nonradioactive LLNA versions, Daicel Adenosine Triphosphate (DA) and bromodeoxyuridine-enzyme-linked immunosorbent assay (BrdU-ELISA); and (5) updated LLNA applicability domain, including pesticide formulations. Recommendations are pending for using the LLNA for potency categorization. The BrdU-Flow Cytometry (FC) method was evaluated, but requires further validation studies before ICCVAM provides formal recommendations. Federal agencies endorsed the updated LLNA, rLLNA, and performance standards in March 2010. ICCVAM final recommendations for the nonradioactive versions of the LLNA were transmitted to agencies in June 2010. At an OECD National Coordinators (NC) meeting in March 2010 the LLNA:DA and LLNA:BrdU ELISA were approved as OECD TGs 442A and 442B, respectively. ICCVAM final recommendations for updated applicability domains of the LLNA were transmitted to agencies in June 2010. These support the use of the LLNA for pesticide formulations; metals (except nickel); substances in aqueous solutions; and other substances/products unless physicochemical properties interfere with the ability of the LLNA to detect sensitizers.

• *Ocular Safety Assessments:* NICEATM-ICCVAM held an international peer review panel meeting in May 2009 and published the independent peer review panel report in July 2009 on the evaluation of 10 alternative methods and approaches. The report supported the routine use of analgesics, topical anesthetics, and humane endpoints and the use of *in vitro* methods for identification of substances not requiring ocular hazard labeling, including the Cytosensor™ (CM). ICCVAM has just forwarded the final recommendations to the HHS Secretary for transmittal to Agencies. Several other methods were not recommended, but were determined to need additional studies for optimization and further validation. Additional studies were recommended for *in vitro* testing strategies [CM, Bovine Corneal Opacity and Permeability (BCOP), and EpiOcular] to assess the eye irritation potential of antimicrobial cleaning products for U.S. EPA hazard classification. ICCVAM recommended discontinuation of the use of the low volume eye test due to issues with accuracy. A Standard Proposal Submission Form was submitted to OECD for a future update of the eye irritation TG to include additional humane endpoints and routine use of analgesics and anesthetics. It was rejected by the OECD Working Group of NCs, which stated that a reduction of pain and suffering in an *in vivo* test method was not seen as a major advancement toward the 3Rs, and could actually encourage expanded use of the *in vivo* test instead of *in vitro* tests, and discourage development of new *in vitro* alternatives. ICCVAM disagreed with this rationale and will resubmit the proposal. NICEATM and the ICCVAM Ocular Toxicity WG, are contributing to the ECVAM-coordinated *Validation of In Vitro Human Reconstructed Tissue Models to Identify Substances as Non-labeled for Ocular Injury Hazards* via liaison members participating on the validation management team.

• *Acute Systemic Toxicity:* A draft OECD Guidance Document on the use of *in vitro* cytotoxicity methods to estimate starting doses for acute oral toxicity studies was prepared in conjunction with the Acute Toxicity WG with ICATM Liaisons. The document was approved at the March 2010 OECD NC meeting and will be published in 2010. NICEATM-ICCVAM is participating in an ongoing validation study of *in vitro* models for human hepatic metabolism and toxicity and an evaluation of 3T3 NRU cytotoxicity assays to classify "non-toxic" substances (LD_{50} > 2000 or >5000 mg/kg) without animal testing.

• *Endocrine Activity Assays:* NICEATM is coordinating the international validation studies of the LUMI-CELL® stably-transfected transcriptional activation assay and the CertiChem, Inc., MCF-7 Cell Proliferation Assay, both of which use human cells (breast and ovarian carcinoma cell lines) with human estrogen receptors (ERs) to detect ER agonist and antagonist activity. These studies are being coordinated through the ICCVAM Endocrine Disruptor (ED) WG and its ICATM liaison members.

• *Genetic Toxicity Assays:* The ICCVAM Genetic Toxicity WG, working with ECVAM and JaCVAM liaisons, is currently contributing to the JaCVAM-led validation of the *in vivo* and *in vitro* comet assays and cell transformation assays. The WG also contributed to revisions to OECD TG 487 on the *in vitro* micronucleus assay, which was recently approved by the OECD NCs.

• *Dermal Safety Assessment Assays:* The ICCVAM Dermal Corrosivity and Irritation WG, with ECVAM and JaCVAM liaisons, is studying the validity of a non-animal assessments of dermal irritation and corrosion potential of chemicals or products. In particular, ICCVAM and partners are contributing to a validation study underway by NICEATM to determine how *in vitro* dermal irritation test methods (i.e., EpiDerm™, SkinEthic™, and EPISKIN™) will classify corrosive chemicals incorrectly identified as non-corrosives by *in vitro* corrosivity test methods.

• *Other Five-Year Plan (FYP) Activities:*
(1) *Priority Test Method Activities*: NICEATM-ICCVAM, with ICATM partners and SOT as a co-sponsor, is organizing the *International Workshop on Alternative Methods to Reduce, Refine, and Replace the Use of Animals in Vaccine Potency and Safety Testing: State of the Science and Future Directions*. It will be held at NIH Headquarters, Bethesda, MD, September 14-16, 2010. (2) *Application of New Science and Technology*: In the High Throughput Screening (HTS) Program, NICEATM-ICCVAM have nominated >900 ICCVAM reference chemicals, will nominate *in vitro* assays for HTS, and would consider promising methods from HTS for validation studies and evaluations.
(3) *Partnerships:* The ICATM MOC, signed in 2009, is a framework for enhanced international cooperation, collaboration, and communication with the four initial participating validation organizations, JaCVAM, ECVAM, the Health and Safety Bureau of Health Canada, and NICEATM-ICCVAM. The newly established KoCVAM is participating initially as an ICATM observer. There are three critical areas of

cooperation: test method validation studies, independent peer review of the validation status of test methods, and development of harmonized formal test method recommendations for regulatory authorities. The goal is to accelerate international adoption of scientifically valid alternative test methods. Since establishment of the ICATM last year, the collaboration has contributed to approval of two nonradioactive LLNA test methods, the updated LLNA protocol and rLLNA by the OECD NC in 2010, and these are now pending formal adoption. The BCOP and isolated chicken eye (ICE) were formally adopted in September 2009. All of these methods were approved much faster than any previous methods proposed to the OECD.
(4) *Fostering Acceptance and Use of Alternative Test Methods:* ICCVAM is organizing a workshop titled *Implementing Alternative Test Methods into Your Regulatory Safety Testing*. SOT is co-sponsoring the workshop, which is scheduled for January 18 – 20, 2011. The objective is to allow participants to gain a practical understanding of available alternative methods and best practices for their consideration and use for regulatory safety testing.

• *2008-2009 Biennial Progress Report:* The report summarizes NICEATM and ICCVAM activities and accomplishments during 2008 and 2009, and is now available to the public.

• *Outreach Activities*: NICEATM-ICCVAM had 8 poster presentations at the 2010 Annual SOT meeting and has proposed two informational sessions for the 2011 SOT meeting, *Moving Innovative Safety Testing Methods from the Bench to Regulatory Approval: Federal Resources for Developers* and *ICATM: Translating Science to Provide Improved Public Health Safety Assessment Tools.*

Dr. Stokes acknowledged the NICEATM staff and Integrated Laboratory Systems, Inc., the center support contractor.

B. SACATM Discussion
Dr. Gwendolyn McCormick asked about communicating with other groups such as the American Association for Laboratory Animal Science and the Institutional Animal Care and Use Committee (IACUC) to promote alternatives. Dr. Stokes agreed with the suggestion and added that he participated in two workshops on alternative methods at the Public Responsibility in Medicine and Research IACUC annual training forum earlier this year. Dr. Linda Toth asked about interactions with trainee programs for toxicology students. Dr. Birnbaum said SOT has a very active postdoctoral training program. She suggested contacting the council member who is the liaison to the student and postdoctoral groups or utilizing their newsletters to provide information about alternatives. Dr. Ehrich said Dr. Stokes spoke at the Colgate student luncheon at SOT three years ago, which was focused on alternatives. Dr. Sharon Meyer suggested introducing alternatives concepts through NIH-funded training grants. Dr. Birnbaum said nothing precludes alternative concepts from being included in a proposal.

Dr. Toth asked about obtaining data that would indicate the extent that alternative tests were being used for submissions to agencies for review. Dr. Stokes said agencies like

the Department of Transportation (DOT), Occupational Safety and Health Administration (OSHA), and CPSC have requirements for testing, but testing data are not routinely submitted to these agencies, so there is no way to know the extent that alternative methods are being used. However, the Food and Drug Administration (FDA) and the Environmental Protection Agency (EPA) do get data submissions. Dr. Jack Fowle said that for the new chemicals program under the Toxic Substances Control Act (TSCA), the EPA does not require testing, but does require information about chemical structure to predict the toxicology. The TSCA is being reauthorized and will include new provisions to try to promote the 3Rs. The pesticides program requires information on different kinds of testing and has criteria, but does not require specific tests. Most of the tests are not alternatives; EPA is trying to encourage them, but does not currently capture information on alternatives. Dr. Fowle said he would provide information on that.

Mr. Gary Wnorowski said an issue is the EPA's acceptance of the LLNA. He alluded to the transmittal regarding the applicability of the LLNA for testing pesticide formulations. He said his company, a contract laboratory, is not running LLNAs on pesticide formulations and questioned when EPA would make a decision regarding its acceptability. Dr. Stokes said agency responses to the LLNA transmittal are due in December and would be posted on the NICEATM website. Dr. Fowle said EPA is accepting the LLNA and EPA's Science Policy Council is currently developing a formal policy. Mr. Wnorowski said his understanding was that EPA was accepting LLNA data on pure chemicals, but not on mixtures. He added that testing ratio is typically 50 mixtures tested for each pure chemical tested.

Dr. Karen Brown said the veterinary biologics industry has progressed with new technologies even before the USDA, which regulates veterinary biologics products. Many industry groups developed *in vitro* assays for vaccine potency testing starting in the early 1970s, but they were not accepted. Now USDA is working with industry to focus on vaccines for diseases like *Leptospirosis*, which uses a lot of animals. The difficulty lies in the standards that have become too high to qualify, requalify, validate, and monitor references for assays. She found it frustrating that now industry is backing away from using *in vitro* assays and going back to animal testing. The regulatory agencies and industry have to work together to find solutions acceptable to both. Dr. Stokes said the September workshop would be an opportunity to further discuss that issue.

Dr. Steven Niemi questioned usage of the words "accept," "endorse," and "require" in the discussion and whether endorsement of an alternative method by a regulatory body gives it greater weight than animal-based assays. Dr. Stokes explained that 31 countries use the OECD international terminology. OECD has a TGs program and when a TG is initially approved, it means that the NCs representing the 31 member countries have accepted it, which is the first step in the formal OECD test guideline adoption process. The U.S. NC is in the EPA Office of Pesticides. The second step is consideration by another committee of representatives from all 31 countries and the third step is formal adoption at the Council level. "Adoption" is the key word for OECD

TGs. When testing is done in any of the member countries using an adopted test method, other countries must accept the data. Within U.S. agencies, the word "accepted" is used for regulatory test methods, e.g., the LLNA. For tests not used for regulatory decision-making, e.g., *in vitro* methods for estimating acute oral toxicity, the term "endorsed" is used, which indicates concurrence with the validity and recommended usage. Dr. Toth said it was important to put in place a mechanism to determine whether validated and accepted tests are actually being used. Dr. Fowle cited Section 4(c) of the ICCVAM Authorization Act, "Each Federal agency . . . shall ensure that any new or revised acute or chronic toxicity test method, including animal test methods and alternatives, is determined to be valid for its proposed use prior to requiring, recommending, or encouraging the application of such test method." He said Federal agencies could engage stakeholders and implement laws that are passed by Congress. Often there are long histories of approaches that are not optimal, but are agreed to by environmental groups, industry, and the public. The EPA has a Pesticide Program Dialogue Committee comprised of people engaged in encouraging alternatives and engaging with various communities. Their webpage lays out a strategic plan and vision.

Regarding Dr. Brown's comments on the USDA, Dr. Kulpa-Eddy explained that some information provided is confidential business information so the USDA cannot publically state the tests that companies are using. Dr. Kreysa said Europe does not have a good system to monitor alternative method usage; however, the alternative tests are required for the protection of experimental animals. With the revised regulations, the member states would be required to report on alternative usage at national level. In 2005 in Europe approximately 12 million animals were used, mostly for research. Dr. Hansen asked if each agency has tracking mechanism for animal usage as a way to gauge progress moving forward. Dr. Stokes responded that only the FDA and EPA could generate those data. Dr. Birnbaum said that information is not forthcoming.

V. Regulatory Acceptance and Availability of ICCVAM-Recommended Alternative Test Methods.

A. Presentation

Dr. Stokes discussed U.S. regulatory acceptance of alternative methods for ACD safety assessments. ICCVAM recommendations were transmitted to Federal agencies on September 18, 2009 on the updated ICCVAM LLNA test method protocol, the rLLNA, and LLNA performance standards. All agencies agreed with ICCVAM recommendations where applicable to their agency. The FDA cited several limitations for use of the LLNA: (1) dermal drug formulations, due to production of false positives compared to guinea pig and human results, (2) drugs/biologics with pharmacodynamic activity to release cytokines, and (3) the rLLNA should be considered on case-by-case basis.

The updated LLNA protocol provides (1) guidance on selection of the highest dose, (2) reduction of minimum dose group size to n = 4, which is a 20% reduction in animal numbers compared to the original LLNA protocol recommended by ICCVAM and

accepted by agencies in 1999, (3) uniform collection of individual animal response data, (4) group housing, (5) guidance on concurrent vs. periodic positive controls, (6) inclusion of the rLLNA procedure, and (7) inclusion of LLNA performance standards. The rLLNA (1) provides a 40% animal reduction per test compared to the updated multi-dose LLNA; (2) should be routinely used as the initial test to determine the ACD potential of chemicals and products except when such substances are suspected of having the potential to produce ACD, and dose-response information is required; and (3) has a low false negative rate compared to the multi-dose LLNA, although all of the false negative substances were borderline weak positives in the multi-dose procedure. Both protocols avoid the pain and distress associated with guinea pig tests. The LLNA performance standards provide the basis for validation and evaluation of proposed test methods that are mechanistically and functionally similar to the accepted reference test method, the standard LLNA, and provide for a much more efficient validation of versions similar to the standard LLNA.

ICCVAM and NICEATM developed and submitted a draft updated OECD TG 429 for the LLNA in 2009 for the updated LLNA test method protocol procedures, the rLLNA procedure, and the LLNA performance standards. These were supported by the ICCVAM Test Method Evaluation Report, Background Review Document, and Peer Review Panel Report. NICEATM, ICCVAM, and CPSC hosted an OECD Expert Consultation Meeting in October 2009. The TG was approved at a NC Meeting in March 2010 and formal adoption is expected in September 2010.

Dr. Stokes discussed international regulatory acceptance of alternative methods for *in vitro* ocular safety testing methods. In 2007 ICCVAM recommended two *in vitro* ocular toxicity test methods as screening tests to identify some substances causing irreversible and severe eye injuries, the bovine corneal opacity and permeability (BCOP) test method and the isolated chicken eye (ICE) test method. These methods were accepted in the U.S. in 2008 and as OECD TGs 437 and 438 in 2009. Using these tests, positive results can be used to classify substances *without* animal testing, which provides for reduction and refinement. These are the first validated *in vitro* alternative test methods for eye safety accepted for worldwide regulatory use.

B. SACATM Questions and Discussion

Dr. Diggs asked if the OECD guidelines are mandatory and how they would impact U.S. companies working internationally. Dr. Stokes clarified that the methods are available for use by any of the organizations in the member countries. Most European countries have animal protection laws similar to the Animal Welfare Act (AWA) and the U.S. Public Health Service Policy on *Humane Care and Use of Laboratory Animals* that require consideration of alternatives before animals are used for testing, research, or education. Rabbits and guinea pigs are covered species under the AWA, so alternative test methods must be considered in the U.S. prior to use of these species for testing if the proposed testing may involve more than slight or momentary pain or distress. Multinational chemical or pharmaceutical companies would not normally be required to retest substances in the U.S. if they were tested appropriately in Europe. Dr. Freeman said data requirements could be met using animal or alternative OECD TGs. The

OECD TG 401 for oral LD_{50} testing for acute systemic toxicity is the only OECD TG to be deleted following acceptance of three alternative methods, including the Up and Down Procedure, which was evaluated and proposed by ICCVAM. Dr. Stokes said there is mechanism to allow acceptance of alternative methods, and deletion of existing methods if the new alternatives can completely replace the existing method.

Mr. Wnorowski agreed that the standard oral toxicity testing is no longer acceptable. However, when there are multiple guidelines that would satisfy a requirement for global regulatory purposes, it must be taken into account whether there is one regulatory agency that will not accept the methods. If that is the case, the non-alternative method must still be used. Dr. Brown inquired whether most companies have the capability of running LLNA tests or doing tissue culture, which may be a challenge if they have only standard animal facilities. Dr. Stokes said for the traditional LLNA, laboratories would need a license for radioisotopes, but the new nonradioactive methods do not require that. He added that companies should be able to see advantages of non-animal methods and move toward finding ways to do them.

Dr. Brown, lead discussant, said awareness of alternatives could be helped by IACUC workshops and training sessions for companies that do studies. It is important to contact industry groups and give presentations to demonstrate how alternatives may be more cost- and time efficient, though much work needs to be done to switch to non-animal tests. To gather data from industry, it is often necessary to submit data, masked to protect proprietary information, to a neutral organization, such as NICEATM. The issue of false positives in *in vitro* tests is problematic.

Dr. Ehrich, lead discussant, said laboratory animal veterinarians on IACUC committees read *Lab Animal*, so articles there would be useful. She suggested putting questions about alternative methods on the certification exam. Veterinarians could provide information to study directors. She agreed that industry is reluctant to submit data, so blinding it or using older data is an option. The FDA's objections to use of the LLNA must be overcome before there is acceptance of it and she suggested combining the LLNA with measuring cytokine release in cell culture systems.

Dr. Meyer, lead discussant, supported previous comments regarding outreach to laboratory animal veterinarians. She suggested incorporating more training of pre- and postdoctoral trainees through training grants and providing lectures on alternative methods. Contract laboratories do much of the work, but there has to be regulatory acceptance of the data and it has to be cost effective. She concurred with Dr. Ehrich regarding the LLNA and questioned whether peripheral lymph nodes may contribute to the false positive responses. She said the BCOP and ICE assays are good illustrations of how alternatives have successfully identified strong irritants and corrosives; these methods should be accepted by industry. The next challenge is refine the techniques to obtain dose response information. Regarding data acquisition, academia is becoming involved in drug development in large consortiums through U01 funding mechanisms. There could be a provision that the data from pre-clinical safety testing should be made available but still have protections of intellectual property.

Dr. Michael Olson, lead discussant, recommended a “hearts and minds campaign,” with more burden put on study directors with the goal of influencing investigators. He advocated informational campaigns with the American College of Toxicology and other groups that specialize in drug and product safety assessment. Some of the alternative methods might be useful for non-toxicologists using screening technologies or members of the American Society for Pharmacology and Experimental Therapeutics. Approaching individual industry investigators, rather than the industry as a whole, may be helpful in obtaining industry data. Dr. Olson questioned the extent of the FDA reservations regarding the LLNA. Any alternative method must be used with full understanding of its limitations. It is an investigator’s prerogative to put methods into or out of scope and to determine the applicability of any method. This is built into the new language regarding adoption of the LLNA, allowing those using the LLNA to recognize the physical characteristics of the substance and to limit utilization of assay.

Dr. Toth, lead discussant, said there is no substitute for peer-reviewed literature for information about humane animal care and use. Concise and comprehensive articles are needed for veterinarians and IACUCs. She agreed with Dr. Meyer that cost savings for alternative methods are crucial. She suggested tapping academia, not just industry, for additional validation information. The issue is having resources to support studies; grant or foundation support is necessary to allow alternative methods to be further developed. She questioned the use of tissues from cows and chickens for ocular testing due to the animals’ unknown background and health status. Eyes from more traditional research animals or sensitized strains would provide more reproducible models.

Mr. Wnorowski, lead discussant, said IACUCs at most institutions are knowledgeable about alternative methods. Test method selection ultimately is driven by regulatory acceptance. He was unaware of any alternative methods currently being discussed that have any dramatic effect on cost. Industry is willing to share data as long as proprietary information is not released, so a careful design of a project to gather data will increase industry’s willingness to participate.

Dr. Han said the Korean FDA has been using IACUCs since 2000. They make the committee aware of the availability of alternative test methods and make recommendations when they review the test plans. They provide information about globally accepted test methods to environmental, agriculture, and toxicology organizations. KoCVAM holds workshops and symposia for industry and academia to present information on alternative methods.

Dr. George Corcoran urged more effective communication of the goals of the 3Rs in non-confrontational ways. IACUCs are a place to start, but they are not always strong advocates of alternative methods. He suggested seeking travel funding to allow IACUC chairs to convene and mandating training in alternative methods and the ethical foundation of 3Rs for PhD and DVM students at NIH-funded institutions. Dr. Diggs suggested webcasting or recorded programs as a part of ongoing IACUC training

because it is difficult to get IACUC chairs together. Dr. Ehrich said veterinary schools provide training in the responsible use of animals in research, which includes training in alternative methods. Dr. Corcoran questioned how much alternative methods are emphasized, how up-to-date the training is, and in what context it is taught.

Dr. Brown said it was important to communicate to upper management the cost effectiveness of *in vitro* assays. Dr. Kreysa said similar issues occur in Europe regarding raising awareness of alternative methods and access to information so ECVAM provides a publically available database. In Europe, training of toxicologists includes information about alternative methods. Regarding obtaining data from industry, ECVAM worked with the pharmaceutical industry to fine-tune a genotoxicity method that produced too many false positives. ECVAM attempts to have dialogues with regulators early in the process of validation. Dr. Snyder questioned whether universities are as engaged as industry in alternatives. Public Health Service (PHS) policy with regard to laboratory animals applies only for NIH-funded activities. NIH has a policy in the Office of Extramural Research on data sharing. Public Law 110 applies to data generated for the creation of regulation or law, i.e., those data have to be made available. However, even with the Freedom of Information Act, intellectual property is protected. Regarding communicating about alternatives, most institutions have a trainer for the laboratory animal program. There is a national organization of those trainers who could be contacted. Every IACUC chair in a PHS-funded institution is in a database maintained by the Office of Laboratory Animal Welfare, which always announces new alternative methods on its listserve.

Dr. Stokes thanked everyone for the suggestions. He said ICCVAM would work to implement the advice about reaching out to study directors, IACUCs, and different organizations.

VI. Assessment of Acute and Chronic Pain in Animals

A. Presentation

Dr. Alicia Karas, a veterinary anesthesiologist at the Cummings School of Veterinary Medicine at Tufts University, said she has a particular interest in animal pain management and assessment. She noted that her remarks would focus on moderate-to-severe pain and distress in animals, with an emphasis on rodents, since they are the most commonly used laboratory animals, particularly in toxicological research and testing. She said she would also concentrate on how best to assess pain and distress; whether through a reliance on training or the effort to establish a more rapid, objective method that may constitute a cut-point for human endpoint use. She recommended two resources for further information: *Anesthesia and Analgesia in Laboratory Animals* (Academic Press, 2008), the first comprehensive textbook in the field, and *Recognition and Alleviation of Pain in Laboratory Animals* (National Academies Press, 2009), an updated version of an earlier National Research Council publication.

Dr. Karas said there is no gold standard for assessing pain or distress in animals, though various researchers have attempted to do so by measuring serum cortisol

levels, new gene expression, physiologic alterations, and through functional imaging; with the exception of the latter, none of these methods are specific for pain. A current industry standard is analgesiometry, a method in which a painful stimulus (e.g., heat or electrical current) is given to an animal, which causes the animal to move away, establishing a threshold or latency that can be measured. Analgesiometric, physiologic, and biochemical measures of pain require careful, controlled conditions, and thus are not suitable as methods for everyday clinical (i.e., cage side) monitoring of pain. There is a great need to establish simple reliable methods for pain assessment in laboratory animals. The ability to understand and assess animals' behavior is key to that type of monitoring, but optimally, it should be done on a scientific basis as opposed to being based on the opinion of individual personnel. The goal in terms of endpoints is to be able to determine when an animal is in *too much* pain or distress. To assess behavior, one can either look at what the animal *is* doing in terms of onset of new behaviors due to pain, such as the adoption of a certain type of posture, writhing, twitching, licking, limping or reactivity, or one can look at what the animal *is not* doing, when the normal activities of daily living such as eating, moving, nesting, or interacting become extinguished because the animal is incapable of doing them. Understanding normal behavior in particular species, and even in specific strains is also an important factor.

The new-onset pain behaviors in rodents have been documented in studies, but they tend to be infrequent, limiting their utility for scoring purposes. For example, the behaviors tend to subside 6-8 hours following surgery; therefore, individual animals must be monitored extensively to document the occurrence of these behaviors. Further, a trained observer is required to discern the subtle effects of pain and distress, making it difficult to assess the duration and magnitude of significant pain. Dr. Karas said that the lack of normal behaviors often manifests as animals becoming more immobile, but not necessarily sleeping. Assessments of the extinguished or reduced behaviors (e.g., weight loss or amount of food in the cage) can give a backward look at how the animal has been feeling—a retrospective measure of the animal's condition over the past 12 to 24 hours. These measures, while useful from a research standpoint, do not support the real time alleviation of pain or distress in the animal.

Dr. Karas introduced the concept of *dynamic pain* as opposed to *static pain*. Dynamic pain such as incision, joint, muscle or bone pain is potentially avoidable by immobility. Static pain from, for example, gastrointestinal cramping, ischemia or burns, is not reduced by immobility. Immobility itself is not the most useful clinical pain indicator; rather watching whether and how the animal can move is more informative. Multiple behavioral cues such as posture and facial expression are used to assess acute pain. The non-painful animal is able to move freely and does "normal" things such as stretching, running, exploring or arranging its environment. Chronically painful animals would exhibit many of the same behavioral cues as animals in acute pain. However, they tend to do the minimum amount of activities. Understanding their normal range of activities is important to allow perception of improvement upon treatment.

Dr. Karas said rodents are fundamentally no different from other animals in terms of the behavioral cues involved with pain assessment, except that in the typical laboratory

there are thousands of them to assess. They are relatively unfathomable compared to other, more familiar animals, and there are very few experts in rodent pain recognition. She described normal mouse behaviors, although the concepts could be applied to other species such as rats, rabbits, or hamsters. The behaviors include group huddling while sleeping, nest building (with depth of nest a quantitative measure), fighting, running in wheels, exploring but not sleeping in tubes, and burying novel items put into cages. Normal mice move quickly, making it difficult to get photos of them without blurring, whereas mice in pain move more slowly. She related normal behaviors in rats as contrasted with mice; rats also stretch and explore, but not as rapidly as mice. Acute and chronic pain-altered behaviors have been studied in rodents, producing a considerable body of literature, supporting her assertion that monitoring multiple behavioral factors is vital to assessing animal pain. She cited a study she had conducted documenting differences in stretch behavior in mice that had anesthesia or surgery as an example of a normal behavior suppressed by pain.

Dr. Karas presented an argument for similarity of behavioral responses to severe pain as well as in other forms of distress, including a possible approach allowing the characterization of pain or distress as intolerable, thus introducing the concept of distress. She described pain and distress as evolutionarily important signals necessary for survival. It would be highly useful to have a simple way to "ask" animals how they feel now, as opposed to using proxy, retrospective measures. This would involve an ability to assess behaviors that are easy to recognize and quantify quickly, to recognize those animals needing special attention, and would be universal for pain or distress. Unfortunately, she said, that ideal is probably unattainable, but detailed, skilled behavior observations such as the assessments of mouse facial expressions described in Langford *et al.,* 2010 (*Nature Methods* 7(6):447) could be of value, albeit likely limited to research applications. Watching briefly for common actions that the animals perform could also be useful, such as nesting in mice or food treat consumption. When they are unable or unwilling to perform such compelling actions, it can be an indicator of pain or distress.

Dr. Karas said there are convincing data to suggest that abnormal mice do not make good nests, and mice that have been treated for pain after surgery make better nests than ones left untreated. She suggested that time to incorporate additional nest material after it has been placed in the cage might be a useful metric for assessing mouse pain and distress, since it is a highly compelling behavior for mice. She mentioned that the time and extent of ingestion of a treat after it has been introduced to the cage might also be useful behavioral indicators. Neither indicator would require any special training of the observer, but would require characterization of the normal state of particular animals for effective comparisons.

Dr. Karas concluded by stating (1) movement and activities are a vital clue to how animals feel; (2) viewing changes in movement and activities may also be a non-specific indicator of distress; (3) if animals have nothing to do, assessing activity levels would not be easy, thus use of environmental enrichments may allow more behavioral clues to

well-being; and (4) any large-scale, robust measure for pain and distress must be simple and involve short observation periods.

B. SACATM Questions and Discussion

Dr. Ehrich questioned whether differences are seen in multiple caged animals versus singly caged animals, in terms of fighting behavior versus an impoverished environment. Dr. Karas replied that male mice tend to fight more, especially when they are given an object to fight over, whereas females fight less, with one typically being the "housekeeper." Singly caged animals tend to make more elaborate nests, lacking other animals to sleep on. Also, they will build nests repeatedly as old ones are removed. Dr. Toth asked about implications for treatment of pain. Dr. Karas said animals thought to be in pain should be treated whenever possible, and that pain should be quantified for those experiments where things could actually be changed. She added that anything that causes people to observe animals more would advance the cause of relieving pain and distress, with an additive effect as observers become more skilled over time. Dr. Bucher asked if there are objective enough measures of pain to set standards or guidelines for termination of an animal. Dr. Karas replied that, as assessment of pain is a multi-dimensional exercise, even in humans; its application to laboratory animals is not adequately characterized. The simple, low-tech methods she had described would be practical and could be investigated easily.

Dr. Diggs, lead discussant, praised ICCVAM and NICEATM for the progress they had made during her four years on SACATM, but expressed frustration that there was still no easy answer to the basic question, "Does this hurt?" Despite increases in understanding of pain and distress in animals, quantitative assessment remains elusive, and substantial gaps remain, with subjective evaluations and informed opinions still predominate. She stressed that this research needs to be a high priority if animals are to continue to be used in research and testing. Resources should be directly earmarked toward research in this area and there should be increased training and instruction at the graduate level, including Internet-based resources. She said this would be one way to better communicate with the international community, since there are many international students enrolled in graduate programs in the US. She said she was in favor of the Small Business Innovation Research (SBIR) approach, as well as other mechanisms to direct funding to research in this area, particularly studies using anesthesia and analgesics with test animals. There should be a focus on interdisciplinary research teams, particularly among the 20 recognized veterinary specialties, many of whom would be well capable of working in the very areas desired. There should be team collaborations of researchers and veterinarians to accelerate progress and outcomes in this area. She advocated expansion of the NTP's award program focused on rewarding reductions in animal use, as well as encouragement of other incentives.

Dr. Steven Hansen, lead discussant, said that work with checklists and behavior exams should be taking place in order to ensure that pre-emptive analgesia has been effective, and that more emphasis should be placed on the administration of pre-emptive analgesia, since it is well known that many of the procedures being used do cause pain.

He felt that the identification and evaluation of objective criteria, particularly the behavioral evaluation techniques, should be a high priority for ICCVAM and NICEATM. He expressed support for the SBIR grant program, and for the existing work that ICCVAM has done in the validation of pain assessment methods. He was disturbed by the OECD rejection of an updated rabbit eye test on the grounds that it might expand use of the *in vivo* test and discourage development of *in vitro* tests.

Dr. McCormick, lead discussant, advocated improved training of veterinary technical staff to reflect a more sensitive, holistic approach to ensuring the physical and psychological wellbeing of laboratory animals. Animals' responses to test agents may relate to how they have been handled, potentially affecting results. Development of assessment criteria should be a high priority, including consistency in the process of understanding when evidence of pain is sufficient to stop a study, or to call for a dosing holiday. Development and validation of a new assay can take many years, and so there is an ethical obligation to refine techniques to reduce the pain and distress involved in still-necessary *in vivo* tests, until they can be replaced altogether.

Dr. Niemi, lead discussant, pointed out that toxicity does not always involve pain, and that pain does not always correlate directly with the magnitude of the stimulus, whether in intensity or duration. The individual organism's ability to perceive and react to pain can influence the effect of pain. Also, reliance on behavioral factors can be tricky, e.g., mice appear to prefer warmer environmental conditions than those tolerated by humans, and so mice might be chronically chilled in rooms that are comfortable for humans, leading to groups huddling together to keep warm, with that being potentially misinterpreted as a behavioral response to pain. He cited two recent studies showing the involvement of genetic variability in pain perception, conserved across species. He felt that those studies might be a starting point for the development of methods to quantify gene activity and influence on pain-related behavior, and for "personalized" approaches to pre-operative and post-operative analgesia based on genotyping of laboratory strains. Methods could be developed to tie specific genes to promoters such as green fluorescent protein to allow direct, easy, quantifiable observation of perceived pain and distress. Although a strong proponent of the SBIR concept in general, in this context he couldn't see an obvious business opportunity yet. He agreed that if there are ways to avoid or alleviate pain in rabbits used for ocular testing, there is an ethical obligation to do so immediately as long as the *in vivo* tests are still in use.

Dr. Toth, lead discussant, recommended characterizing the tests and developing standardized interventions based on the expectation or likelihood that they would induce pain. Assuming on a species basis that particular animals would be likely to experience pain as a result of a given process and intervening prophylactically would obviate the need for highly trained observers. She noted that she does not regard checklists highly; it would be more effective to determine the key variables associated with a particular test and test population. She expressed concern that many scientists would be interested in pursuing the topics being raised in the SBIR announcements, but would be unlikely to participate due to not having an entrepreneurial nature. She predicted that there would be more applications if it were an R01 or R21 program. She advocated

research to determine pain-related markers that could be used as stop-test endpoints, ideally before severe pain developed. She agreed with other discussants' reactions to regard the OECD response to ICCVAM's recommendation.

Dr. Ehrich questioned whether distress interfered with toxicology testing. Dr. Bucher replied that the NTP does what it can to prevent such confounding factors, being interested in studying toxicity, not pain. Dr. Meyer expressed concern that two issues were being muddled in the discussion: pain and distress as the results of a procedure in a testing protocol, versus pain and distress as parts of an animal's response to exposure to the toxicant. She considered body weight change a valuable measure of pain, and was concerned about the use of pre-emptive analgesia potentially shifting dose-response curves. Dr. Bucher agreed that body weight change is a sensitive endpoint, but its relationship to pain is unknown, and if pain and distress precede body weight change, researchers want to know that ahead of time, regardless of whether it shifts dose-response curves. Dr. McCormick concurred that an objective metric of pain and distress would be preferable to relying on body weight change. Dr. Han felt that KoCVAM would give a more positive response to the ICCVAM proposal regarding alleviation of pain in ocular testing.

Dr. Freeman raised the question of whether prioritizing pain assessment activities should go to a vote, and asked Drs. Stokes and Bucher to comment. Dr. Bucher was hesitant about asking for a vote, noting that when the questions were prepared, this one was related to how advanced the field was and whether it was even "ripe" enough to become a high priority ICCVAM action item, or more likely to gain from being put out to the extramural community for further development. Dr. Stokes added that NICEATM does accept nominations, and that if the committee felt that this should be a high priority for NICEATM activity, that type of guidance would be helpful. He said a vote establishing consensus from the committee about making it a high priority would be welcomed.

Dr. Toth said SACATM appears to support the pursuit of basic science questions, whereas the role of ICCVAM has been more focused on the development of specific tests, as opposed to basic research in the area of animal pain and distress; it may be more effective to preserve that more focused viewpoint. Dr. Stokes agreed that in order to have an actual impact on decreased animal pain and distress, there is a need make with specific recommendations, as was done with the alternative eye irritation tests. There is a continuum of activity needed, from identifying where new ways of doing things are needed, identifying knowledge gaps that need to be addressed by research, developing and validating new strategies or approaches, and consideration of their acceptance for regulatory purposes in order to have an impact in terms of the 3Rs. NICEATM and ICCVAM work to identify needs at all levels of that continuum. Dr. Freeman again asked the committee about voting on the concept. Dr. Corcoran said he had no objection to that idea, but would be more comfortable if it were placed in the context of the entire range of activities being presented to SACATM, i.e., that placing a priority on one element would necessitate placing a lower priority on others. He also wanted to see a deeper analysis of the likelihood of moving the issue forward

significantly. Dr. Brown agreed, but would want to see the focus on areas of actual accomplishment. In the veterinary biologicals industry, the endpoint is typically the death of the animal, and any earlier endpoints would be very much welcomed. Dr. Freeman said there seemed to be agreement on the committee that it should be a general priority, and asked Dr. Stokes if he felt that his organization needed more than that, in the form of a formal nomination or a vote. Dr. Stokes said that a nomination is normally fairly specific and involves recommendation of a specific activity.

VII. Federal Agency Research, Development, Translation, and Validations Activities Relevant to the NICEATM-ICCVAM Five-Year Plan

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

Dr. Donna Mendrick, Director of the Division of Systems Biology at the FDA's NCTR, provided an update on their work that supports the NICEATM-ICCVAM FYP. She showed the organization of the NCTR scientific divisions, which are led by Dr. William Slikker, Jr. and provided examples of projects on which her division is working:

•*Computational/Bioinformatics and In Silico Toxicity:* ArrayTrack™, an integrated solution for managing, analyzing, and interpreting microarray gene expression data, is publically available and is being adapted for other high-dimensional data applications such as single nucleotide polymorphisms (SNPTrack). It provides a compendium of rich functional information about genes, proteins, and pathways for biological interpretation. MicroArray Quality Control (MAQC) is an ongoing international consortium to evaluate emerging microarray technology applications. It produced guidance for interpretation of microarray experiments and appropriate methods that can be used to identify and qualify biomarkers. MAQC is currently evaluating the next generation sequencing technologies. NCTR is using several *in silico* toxicity approaches including (1) Quantitative Structure-Activity Relationships (QSAR)/SAR, which utilizes existing toxicological data endpoints and structural chemical information; (2) Quantitative Spectra Data-Activity Relationships (QSDAR)/SDAR which utilizes a patented computational procedure using existing toxicological data endpoints and empirical structural quantum mechanics data of NMR spectra; and (3) docking, which utilizes 3D information of two molecules (e.g., drug and protein) to predict interactions. Some *in silico* projects include development of new predictive models for identification of ED compounds, polypharmacy to build models to identify drug interactions that inhibit two drug-metabolizing enzymes, and prediction of hypersensitivity to drugs.

•*New Model Systems:* The NCTR is beginning to test developmental toxicity using zebrafish and stem cell lines. Zebrafish have anatomic and genomic similarity with humans and have short developmental cycle times and highly reproducible responses. Data on developmental toxicity of hundreds of chemical compounds show very good concordance with human data. The Embryonic Stem Cell Test (EST) assay is being used to evaluate chemical effects on developmental biology. Its overall accuracy is ~ 78%, but is reported to be more inaccurate when drugs are tested. The NCTR is testing some additional stem cell lines to improve accuracy and make the assay more

automated. The NCTR is testing for genetic toxicity using the *Pig*-a Gene Mutation Assay, an *in vivo* test that measures a mutation in X-linked gene by assaying red blood cells. The *Pig-a* gene is coded in multiple species so cross-species evaluations (including rodents and man) are possible and repeated measures can be performed. The responses are cumulative with repeat dosing, so the assay lends itself to incorporation into standard 28- and 90-day toxicity studies.

•*New Approaches to Identify Biomarkers of Disease and Toxicity:* The NCTR is working to develop biomarkers for hepatotoxicity because current biomarkers are insufficient. Biomarkers are needed that identify susceptible individuals, predict development of injury before functional impairment, and identify liver repair. Current projects include utilizing model drugs and chemicals and employing genomics, metabolomics and proteomics with mechanistic and modeling analyses to find biomarkers; building a knowledgebase by combining mining of public domain, genomics and cell-based assays; and *in silico* modeling to study the propensity to develop idiosyncratic hepatotoxicity.

•*Bio-Imaging:* The new facility at the NCTR currently has MicroPET™ and Biospec® magnetic resonance imaging (MRI) capability. Imaging is multiparametric, allowing analysis of the same animal or human over time to collect information and perform quantitative measures. Since these types of imaging are a non-invasive techniques, they may contribute to the discovery of translational biomarkers. One NCTR pilot study is underway to use bioimaging to detect early functional impairment of the liver.

Dr. Mendrick closed by stating that the efforts at NCTR/FDA are focused on research needs for science-based decision making and on technical innovations to help speed FDA-regulated product review and safety assessment.

Dr. Eugene Elmore asked if NCTR's 'omic approaches and software account for interactions within the cell, active complexes of proteins/enzymes, and the crosstalk between pathways. Dr. Mendrick said dealing with these interactions is major problem for everyone and understanding them is an ongoing process. Dr. Niemi asked about the Predictive Safety Testing Consortium, which studied renal biomarkers. Dr. Mendrick said the Consortium comes out of the Critical Path Institute, which has a number of WGs. Her division at NCTR is involved in the liver WG and is careful not to duplicate studies. Data will be put in the public domain and the compounds that will be studied will be those that induce idiosyncratic and unique responses.

B. NIEHS/NTP High Throughput Screening Initiative

Dr. Raymond Tice, Chief of the NTP Biomolecular Screening Branch, mentioned the 2007 National Academy of Sciences report on toxicity testing using *in vitro* in human cells or cell lines and high throughput robotic assisted methodologies. The Memorandum of Understanding (MOU): *High-Throughput Screening, Toxicity Pathway Profiling and Biological Interpretation of Findings* was signed in February 2008 by the Tox21 partners: NIEHS/NTP, the NIH National Human Genome Research Institute/NIH Chemical Genomics Center (NCGC), and the U.S. EPA Office of Research and

Development. The FDA will be joining as a partner under a new MOU. The goals of Tox21 are to (1) prioritize chemicals for more extensive toxicological evaluation; (2) identify mechanisms of chemically-induced biological activity to characterize toxicity pathways, facilitate cross-species extrapolation, and provide input to models for low-dose extrapolation; and (3) develop predictive models for biological response in humans. The Tox21 WGs are Assays & Pathways, Compounds, Informatics, and Targeted Testing.

The NCGC conducts quantitative high-throughput screening (qHTS) of dimethyl sulfoxide (DMSO)-soluble chemicals in a 1536-well plate format to create 15-point concentration-response curves. Dr. Tice reviewed the criteria and requirements for qHTS assays. Assays can be nominated for consideration to the NTP or EPA by anyone; if nominated to the NTP, they are first reviewed by HTS Faculty and then by the Tox21 Assay WG with selection based on appropriateness in terms of practicality and relevance. Characterization at the NCGC is based on how well the assay performs (1) in the 1536-well format; (2) when tested against a set of compounds with known activity for the endpoint in question, taking into account intra- and inter-run reproducibility; and (3) when used to screen a larger compound library. For Phase I, the NTP provided 1408 compounds and EPA provided 1642 compounds, with a 400 compound overlap. Phase I included assays for apoptosis, cell viability, DNA damage, epigenetics, nuclear receptors, and stress response pathways.

EPA's ToxCast™ Program is the research program of EPA's National Center for Computational Toxicology, which addresses the chemical screening and prioritization needs for EPA and generates biological fingerprints. Data are released via the Aggregated Computational Toxicology Resource (ACToR), which brings together data from >500 sources on >500,000 environmental chemicals. There was phased development of ToxCast™: Phase I consisted of 320 pesticides, Phase II consists of ~700 compounds includes nanomaterials, data-rich chemicals, drugs from pharmaceutical companies that have failed in clinical trials, and immunotoxicants/dermal sensitizers (to be used for signature development).

For the Phase II 10K compound library, Tox 21 started with ~120,000 compounds and used exclusionary criteria to create a list of ~11,000 compounds that are defined compounds with known structures and physical/chemical properties. The proposed initial Phase II NCGC screening strategy has a goal of prioritization for more comprehensive testing. The first set of assays should provide broad coverage for the ability of compounds to affect key signaling pathways and cellular homeostasis. The initial focus is on nuclear receptors and stress response pathways.

The NTP Phase I library at the NCGC included 45 ICCVAM endocrine reference compounds that were tested across 12 nuclear receptor assays, including estrogen and androgen receptor transcriptional activation assays in agonist and antagonist mode. NICEATM has nominated ~860 compounds for inclusion in the Tox21 10K library and nominated LUMI-Cell®, an ER transcriptional activation assay, for use at the NCGC.

The NICEATM and ICCVAM Immunotoxicity WG plans to nominate HTS skin sensitization assays for evaluation at the NCGC.

In related work, Tox 21 is conducting human and rodent susceptibility studies at the NCGC. With University of North Carolina collaborators, they have conducted qHTS cytotoxicity and caspase 3/7 studies on ~78 lymphoblastoid cell lines using 240 toxic compounds to evaluate for differential responses and to conduct genome-wide association studies. The Host Susceptibility Branch is working to provide lymphoblastoid cell lines, embryonic fibroblasts, and/or primary hepatocytes from ~35 mouse strains to evaluate differential sensitivity. Tox 21 is also evaluating the applicability of new 'omics technologies to NTP archived tissues. Dr. Tice explained some of the critical issues in the Tox21 work: incorporation of metabolism into high throughput screening, statistical versus biological significance, extrapolation of *in vitro* concentration to *in vivo* dose, concentration versus duration relationships, non-adverse versus adverse effects, reversible versus irreversible effects, interactions between chemicals, and interactions between cells and between tissues.

In 2010 NIEHS awarded five SBIR/Small Business Technology Transfer (STTR) Contracts: *Development of Mid to High-Throughput Toxicological Tests Using Model Organisms, Integrated Prediction Systems to Support Environmental Toxicological Assessments, Incorporation of Metabolism into Quantitative High Throughput Screening Assays, Development of Quantitative High Throughput Screens for the Detection of Chemicals That Modulate Gap Junction Intercellular Communication,* and *Monitoring In Vivo Gene Expression Changes After Exposure to Toxicants in Caenorhabditis elegans.* For 2011 NIEHS has approved three SBIR/STTR concepts: *High Throughput Screening for Reactive Oxygen Species Mediating Toxicity, In Vitro 3D Tissue Models for Toxicity Testing*, and *Application of 'Omics Technologies to Rodent Formalin-Fixed, Paraffin Embedded Tissue Samples.*

Dr. Corcoran asked about using human cells and tissues. Dr. Tice some assays are independent of the source of the cells, but for other assays, the cell types are critical. Some chemicals are universally cytotoxic, whereas other chemicals are effective in some cell types but not others. Tox21 was unable to determine a pattern that would indicate what cell type would respond to what chemical, though there were more hits with rodent cells than with human cells. Tox21 is now adding a cytotoxicity assay to assays that use a reporter gene. The issue of cell type would not be resolved in the short term, though the RegenMed *in vitro* 3D liver system may be useful to make the needed comparisons.

Dr. Elmore asked about delivery mechanisms that can provide concentrations that are relevant to the exposure to the tissue. Dr. Tice said that the ability to extrapolate from *in vitro* concentration to *in vivo* dose is a critical issue and that efforts are underway at the NTP and EPA to develop methods for achieving this goal. In prioritization, chemicals are selected that hit multiple targets at a relatively low concentration, or that hit a single target of special importance. For prediction models, extrapolation is critical. The concentration in *vitro* is usually the concentration added to the media, not the free

concentration, because of protein binding. The EPA and the Hamner Institute have assessed the top 40 chemicals in ToxCast™ Phase I, calculated the concentration *in vitro* and used kinetic analysis to estimate the dose *in vivo* and compared that to *in vivo* data on those compounds. For some chemicals, the AC_{50} concentration *in vitro* overlapped with the *in vivo* levels, which would help with prioritizing chemicals. Dr. Elmore suggested the NTP and other researchers should collect and publish blood levels of chemicals from animal studies to allow correlations to be made between *in vitro* concentrations and exposure. Dr. Bucher said the NTP does generate blood level information on most of the chemicals that are studied.

Dr. Elmore said 24-72 hour exposures in cell culture would allow for the detection of progression of effects or reversal of effects, but the HTS system would not collect that information. Dr. Tice said there is an assay system for measuring the time course of cytotoxicity, but the assay is high throughput. Also, a high content screener has been incorporated into the NCGC to allow for follow-up studies, such as ones that monitor the kinetics of an effect. Tox21 is looking at strategies, going from high throughput to lower throughput in *C. elegans* and zebrafish embryos to obtain the spectrum of data needed. Dr. Meyer asked for the basis for 92 µM limit of the dose range. Dr. Tice explained that the concentration was practical when starting from a 20 mM stock solution, but a limitation is the solubility of the chemical. Responding to Dr. Meyer's question on the 3-fold signal-to-background ratio, Dr. Tice said all assays have a positive control and the 3-fold difference relates to the positive control.

C. Validation of Endocrine Disruptor Test Methods

Dr. Warren Casey, Deputy Director of NICEATM, provided some historical perspective on ED methods. In1996 Food Quality Protection Act (FQPA) and the 1996 Amendments to the Safe Drinking Water Act (SDWA) require EPA to: *Develop a screening program, using appropriate validated test systems and other scientifically relevant information, to determine whether certain substances may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or other such endocrine effect as the Administrator may designate.*

To address this issue, EPA has developed a two-tiered screening and testing process. In Tier 1, EPA hopes to identify chemicals that have the potential to interact with the endocrine system. In Tier 2, EPA will determine the specific effects caused by each ED and establish the dose at which the effect occurs. As a charter member of ICCVAM, EPA is following the interagency validation framework in the development and refinement of assays according to the 3Rs. EPA will use these validated methods or assays to identify and characterize the endocrine activity of pesticides, commercial chemicals, and environmental contaminants, specifically in relation to estrogen, androgen, and thyroid hormones.

NICEATM became involved with EDs in 2000 with the EPA nomination of *in vitro* ED test methods to ICCVAM for evaluation of their validation status. In 2002 the Expert Panel Report and background review documents were published on the *Current Status of In Vitro Test Methods for Detecting Endocrine Disruptors.* ICCVAM's Final Report: *ICCVAM Evaluation of In Vitro Test Methods for Detecting Potential Endocrine*

Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays was completed in 2003 and updated in 2006, reported that none of the test methods were adequately standardized and validated. ICCVAM and Expert Panel developed a list of 78 reference chemicals and standard protocol components to be used in subsequent evaluations of ED methods.

There is intense interest in ED compounds as evidenced by the February 2010 testimony by Dr. Birnbaum to the U.S House of Representatives Committee on Energy and Commerce Subcommittee on Energy and Environment addressing *Endocrine Disrupting Chemicals in Drinking Water: Risks to Human Health and the Environment.* Dr. Birnbaum's testimony stressed three relevant points: (1) EDs evoke their effects at extremely low levels, meaning the test methods have to be extremely sensitive; (2) EDs create a broad range of health effects, e.g., cancer, diabetes, and early puberty, and (3) an integrated testing strategy is essential to determine ED effects. Dr. Casey gave other examples of EDs in the media, such as bisphenol A and oil dispersants in the Gulf of Mexico.

Dr. Casey briefly reviewed the endocrine system and said ICCVAM is focusing on thyroid hormones, androgens, and estrogens. There are two known ERs, ER-*alpha* and ER-*beta*. When interacting with estrogenic substances they form hetero- or homodimers, which bind to estrogen response elements in DNA and activate specific genes. The proteins produced as a result of gene expression then create the cellular response, both of which are measured by current downstream assays. The Lumi Cell® ER Assay, developed using SBIR funding, uses a human ovarian cancer cell line in a 96-well format plate. The assay is being validated in a four phase international study organized by NICEATM, ECVAM, and JaCVAM using 78 coded ICCVAM-recommended test substances. Laboratory work has been completed, data analysis is currently underway, and U.S. and OECD acceptance is anticipated in 2012. The MCF-7 proliferation assay, developed with NIEHS-sponsored SBIR funding, uses human breast adenocarcinoma cell line to measure ER activity in a partially automated system. It is in a four phase international validation study organized by NICEATM, KoCVAM, and JaCVAM, and is testing 52 coded ICCVAM-recommended test substances. Testing will be completed in 2010; U.S. and OECD acceptance is anticipated in 2012.

D. SACATM Discussion

Dr. Corcoran, lead discussant, commended ICCVAM and NICEATM for assembling the impressive FYP. He was complimentary of the degree of collaboration and cooperation among agencies. The FYP supports an approach and provides a blueprint to achieve strategic objectives. The FYP poses some challenges; it becomes a static snapshot and much has changed between 2007and 2010. All plans are necessarily vague in the later years with greater granularity in the first years. Decisions regarding priorities need to be made with regard to the strategic plan. Future efforts would benefit from having a shorter phase of two years with significant granularity that included goals, milestones, and metrics, and that included a two-year progress report. It would be helpful to target two major audiences, agencies and end users of the technology; a future plan should have two areas clearly delineated from those points of view so that activities related to

specific audiences could be evaluated. He said Dr. Tice provided a perfect description of the challenges of trying to incorporate toxicity testing in the 21st century. Much progress has been made in the last 12 months. Focusing on the evaluation of pain and humane endpoints would change the direction of the strategic plan. He suggested revaluating the priorities on an annual basis. Dr. Stokes said SACATM had been involved in strategic planning in the past and ICCVAM would welcome input on updating the plan.

Dr. Ehrich, lead discussant, said some of the key challenge areas are progressing faster than others, such as in developing partnerships, where there has been great progress. The presentations evidenced great advances in developing new science and technology. The USDA has many challenges involving biologics and vaccines and she encouraged continuing multiagency involvement and assessing the needs of regulatory agencies, since they work to protect human and animal health. She stressed the importance of having methods accepted by regulatory agencies, understanding the important endpoints, determining what data can come from *in vitro* sources, and what contributes to the reluctance of accepting alternative models.

Dr. Elmore, lead discussant, lauded the presenters on their research and progress and concurred with Dr. Ehrich regarding the program. Regarding acute systemic toxicity assays, he previously recommended developing stem cell lines to provide pools of stem cells to be used in the future. Testing cannot be designed for just one species, but he suggested more emphasis be placed on stem cells because they are providing models for different organs. He recommended building on the stem cell research to provide a second tier of tests beyond HTS. It would be helpful to incorporate NTP data on animal blood levels when assessing compounds in humans.

Dr. Meyer, lead discussant, said she supported ICCVAM's formation of the research and development WG. She agreed with Dr. Elmore regarding blood level data because when the *in vitro* methods reach a validation stage, a metric would be needed to bridge between *in vitro* and *in vivo* platforms. When data from the 'omics techniques go into a comparative phase for dose response, the data are filtered and compressed and dynamic ranges need to established. This requires some statistical exercises on how the determination for a hit is made. It is necessary to collect information on whether the agencies are implementing the alternative methods. Alternative methods have been successful in categorizing severe hazards, but need to be fine tuned to better predict hazard over a wider range. She suggested more emphasis on statistical evaluations to get dose response information to align with *in vivo* data. Chemicals chosen for validation should have blood level biomarker data from animals. NICEATM-ICCVAM can strengthen their leadership role by helping fill the data gaps for TSCA. She questioned the 92 µM cut-off point used for ToxCast™, which would limit the data's use for risk assessment and developing regulatory standards. Regarding the ED program, she suggested using statistical techniques to reduce the number of animals used in traditional *in vivo* tests, in addition to validating the two *in vitro* assays.

Dr. Qu, lead discussant, was very impressed with the progress made by ICCVAM and NICEATM. She advocated strong collaborations and teamwork among several fields including statistics, computer science, biology, and toxicology. She said MRI data is much more accurate than just observing animals and she questioned how all the technology is being used for the 3Rs. She suggested emphasizing to Federal agencies the cost savings and ability to get accurate, quantitative data by using *in vitro* testing.

Dr. Niemi was impressed with ICCVAM's efforts but questioned using cells from 35 mouse strains. Dr. Tice explained that NTP's host susceptibility program is studying the relationship between genetic background and disease in the 35 strains. Using cells from those strains will assess whether the *in vitro* technology would identify differences in sensitivity based on a pathway analysis. The animal models would then be tested for high- and low-responders.

Dr. Corcoran said he found the ICCVAM Biennial Report very valuable and suggested adding two sections under each strategic goal, placing all work within the strategic context and closing with a strategic reflection. This should help with tying spending to strategic planning, prioritization, and increasing impact. He suggested convening biennially the key individuals in the 15 agencies. Drs. Stokes and Bucher agreed with the suggestion. Dr. Bucher said there was initially skepticism regarding HTS, so it wasn't mentioned in the FYP; however, it is moving forward and has a lot of promise for the prioritization of chemicals in the TSCA reauthorization. He said another area of HTS involves analysis to relate genes and disease pathways. Intersection of those two activities is critical because it all has to fit together to bring out the total potential of the HTS program. He agreed that relating the PK information to human blood levels is important in understanding toxicity pathways. Dr. Bucher expressed support for the NTP providing blood level information.

Dr. Brown agreed that the Biennial Report was valuable and asked about its distribution. Dr. Stokes said its publication is announced in the Federal Register, by trade organizations, and on various listserves. He welcomed suggestions for further distribution. Dr. Brown said many in the veterinary biological community are not aware of NICEATM and ICCVAM.

VIII. Current Issues in the Validation of Alternative Methods for Assessing Chemically-Induced Eye Injuries VIII. Current Issues in the Validation of Alternative Methods for Assessing Chemically-Induced Eye Injuries

A. Presentations

Dr. Stokes presented an overview of two technical issues that arose during a recent ICCVAM and NICEATM evaluation of alternative methods used to identify chemically-induced eye injuries. The issues are 1) The *Minimum Number and Proportion of Animals with Eye Injuries for Classification of a Chemical as an Eye Irritant*, and 2) *Reduced Eye Hazard Labeling Resulting from Using GHS Criteria Instead of U.S. Classification Criteria*. Dr. Stokes briefed the committee on the importance of eye safety testing and eye hazard labeling, and the larger context of the issues to be

discussed. Two million eye injuries occur annually in the U.S., representing a significant burden in terms of health care costs, lost workdays, and temporary and permanent disability. Chemicals and compounds are the third most common product category associated with eye injuries, accounting for 13% of all eye injuries, or an estimated 260,000 injuries annually. The EPA, CPSC, FDA, and OSHA require eye safety testing and labeling of potential eye hazards to provide safety messages to help prevent injuries.

Dr. Stokes reviewed the EPA's Eye Injury Hazard Categories and Labeling Requirements, which are based on the rabbit Draize test, which is currently the standard test method used for all worldwide eye hazard classification and labeling. Category I, labeled DANGER, involves severe eye damage and eye injuries lasting more than 21 days. Category II, labeled WARNING, involves injuries that clear within 8-21 days. Category III, labeled CAUTION, flags for injuries lasting seven or fewer days. There is also a Category IV, with an optional CAUTION label, involving injuries that resolve within 24 hours.

All regulatory hazard classification systems use the same scoring system for the nature and severity of lesions. However the classification criteria used to determine whether a chemical would require hazard labeling, and the appropriate hazard category, vary widely among U.S. agencies, nations, and international organizations. These classification criteria are based on the frequency, nature, severity, and duration of the eye injuries. The EPA has its own system, while CPSC and OSHA use a system based on the Federal Hazardous Substance Act (FHSA) regulations. A United Nations Globally Harmonized System for the Classification and Labeling of Chemicals (GHS) was originally published in 2003 and last revised in 2009. GHS is currently under consideration for implementation by U.S. agencies. ICCVAM evaluates new test methods for their accuracy for correctly classifying the hazard potential of chemicals for each of the U.S. and international hazard classification schemes. This involves calculating sensitivity, specificity, false positive rates, and false negative rates for each of the classification systems by comparing the *in vitro* predicted hazard category to the assigned hazard category in each system resulting from the *in vivo* reference test method.

However, two issues arose during a recent evaluation. First, ICCVAM encountered difficulty in assigning and classifying chemicals as eye hazards using FHSA classification criteria when it recently reviewed available *in vivo* reference data. This arose due to the fact that many chemicals would have required additional animal testing to assign a definitive FHSA hazard category, but such testing was not conducted. NICEATM, in consultation with the ICCVAM Ocular Toxicity WG, performed analyses to identify FHSA hazard classification criteria that could be used to classify these substances without additional testing, and criteria that could be used to classify substances when only 3 animals are used as recommended in several current test guidelines for *in vivo* ocular safety testing, instead of six to 18 as required in the current FHSA regulations.

The second issue NICEATM found during its analyses was that one *in vitro* method correctly identified chemicals that would not require eye hazard labeling using the GHS system, but failed to identify several chemicals as eye hazards that are currently classified and labeled as eye hazards in the U.S. Further investigation revealed a significant discrepancy between the GHS eye hazard criteria and current EPA, OSHA, and CPSC eye hazard classification criteria. The GHS criteria significantly reduce labeling of potential eye hazards compared to current U.S. criteria, with over 30% of chemical eye hazards no longer identified as hazards using GHS criteria.

Dr. Stokes reviewed the current *in vivo* rabbit test, including how rabbit eye injuries are scored in cornea, iris, and conjunctiva tissues. In the cornea, there is a 4-point scale for scoring positive lesions. In the iris, there are just two scores for positive lesions. In the conjunctiva, redness is scored as a 1 for minor redness, but only a score of 2 or 3 for increasingly severe lesions are considered as positive scores. Chemosis, or conjunctival swelling, is scored as a 1 for minor swelling, but only a score of 2-4 for increasingly severe lesions are considered as positive scores.

Dr. Joseph Haseman presented data regarding numbers of animals used in ocular testing. The FHSA regulations require a classification system involving up to three tests, each involving six animals. If the first test is inconclusive, there is a second test, and a third if the second is also inconclusive. Thus, up to 18 animals may be used with this approach. However, current best practices for eye irritation/corrosion tests normally use only up to three animals, so a comparison was needed to ensure that the smaller sample size would retain the appropriate sensitivity and specificity compared with the larger sample size tests, with the same level of hazard labeling as the current regulatory requirement (16 Code of Federal Regulations [CFR] 1500.42). His view was that the current FHSA sequential testing strategy is not very protective. He stated that he would show that a decision procedure based on just three animals is on average at least as protective as the current sequential procedure using up to 18 animals. Going over the sequential procedure, he showed that it contained some questionable aspects. For example, a positive response in 1 out of 6 test animals would be interpreted in three different ways in the three sequential tests, despite the fact that biologically the response is equivalent; in the first test, it's considered negative. In the second, it's considered inconclusive. In the third, one of six is considered positive, and the substance is labeled an eye hazard. He further showed that a positive interpretation, with labeling, could be generated by responses in as few as 4 of the 18 (22%) animals. In another scenario of the decision sequence, as many as 5 of 18 responses would result in no labeling. Dr. Haseman showed a chart depicting the number of animals required to assign an irritant classification under the sequential testing strategy. At the stage of the test, the minimum number of positive animals was four of 18, or 22%, but the maximum number of negative animals for a decision *not* to label was five of 18 or 28%. Ultimately, the sequential testing strategy appears to be confusing and may result in anomalous findings.

Dr. Haseman presented the results of his calculations comparing the sequential testing strategy with two versions of the three-animal strategy, one of which involves a positive

threshold of just one animal, the other requiring two or more positives for a decision to label. He called these Strategies 1 (sequential), 2, and 3. To effectively compare the protective value of the strategies, he looked at a range of underlying response rates. He found that Strategy 3 (which he identified as being roughly equivalent to the GHS system) would identify far fewer irritants than 1 or 2. Strategy 2, with its zero tolerance for positives, would be far more protective than the others, in that it would label more often based on lower underlying response rates. Ultimately, his study showed that Strategy 2, using a criterion of at least one positive animal in a three-animal test, would be at least as protective as current FHSA testing requirements, and that changing to that strategy would result in a saving of up to 83% fewer animals. Thus, he concluded, the three-animal strategy has much stronger basis for its use compared to the current sequential testing approach.

Dr. Stokes then presented the conclusions of the NICEATM analysis regarding Issue 2, the reduction in eye hazard labeling that would result from using the GHS criteria instead of U.S. criteria. NICEATM compiled and analyzed actual *in vivo* rabbit eye test results for a total of 262 chemicals from two databases, and calculated and compared EPA, FHSA, and GHS hazard classifications for each substance. Of 168 chemicals considered to be eye hazards under EPA classification criteria, 59 (35%) would not be so labeled as hazards under GHS. Of the 73 chemicals labeled as EPA Category III eye hazards, 57 (78%) would not be labeled under GHS, while two EPA Category II chemicals would not be labeled under GHS. Dr. Stokes presented data regarding the severity and duration of the eye injuries presented by the 59 chemicals that would not be labeled under GHS criteria. Forty-two % of the GHS “not labeled” chemicals produced grossly visible corneal and/or irideal injuries expected to interfere with normal vision. Twenty-five % of the chemicals had visible corneal and irideal injuries at 48 hours after application, and 19% had visible injuries at 72 hours post-application. Using the FHSA criteria, up to 30% of FHSA eye hazards would not be labeled as ocular hazards under GHS. Ultimately, using GHS criteria resulted in no hazard labeling for 30-35% of substances currently labeled as eye hazards under U.S. Federal regulations.

U.S. regulatory agencies are currently considering adoption of the GHS eye hazard criteria, and OSHA issued proposed rule making in 2009 to adopt the GHS criteria. Dr. Stokes emphasized that the GHS was negotiated with and emphasizes the principle that “the level of protection offered to workers, consumers, the general public, and the environment should not be reduced as a result of harmonizing the classification systems.” However, there are no data to support that the reduced labeling for eye hazards that will result under GHS would not reduce the level of protection of workers and consumers provided by current U.S. regulatory hazard labeling.

He reiterated the main reasons why GHS criteria reduce eye hazard labeling compared to U.S. regulations. First, the minimum number and proportion of animals required to classify a substance as an eye hazard differs significantly, with GHS requiring that a minimum of two out of three animals must have positive responses, compared to only one out of three in U.S. requirements. Secondly, the GHS requires a greater severity of

eye injury as the minimum criteria for a positive response compared to the threshold for a positive response in U.S. requirements.

Dr. Stokes said there is a process for updating the GHS, which appears to be necessary to achieve hazard labeling that will support the GHS principle that the level of protection should not be reduced by the harmonization. Three proposals have been developed for optional or revised GHS labeling criteria that can provide hazard labeling at least equivalent to that provided by current U.S. regulations and therefore avoid the reduction in hazard labeling. These include: (1) adding an optional category for countries wishing to maintain their current level of hazard labeling; (2) retaining the current GHS criteria for Category 1 and 2A, but revising the current optional GHS Category 2B criteria to classify substances as ocular hazards based on positive ocular injury score in a least one animal (vs. the current two or more) at any of the three daily time points (vs. a three-day mean score), and (3) revising the current GHS Category 2 to classify substances as ocular hazards based on a positive ocular injury score obtained in at least one animal (vs. two or more) at any of the three daily time points (vs. a three-day mean). Any of the three proposals would identify all 59 EPA and FHSA eye injury hazards not currently classified by GHS.

Dr. Freeman asked Dr. Stokes how this particular issue is relevant to SACATM, because this agenda item does not appear to be concerned with validation or adoption of alternative test methods, but rather, is about the criteria for hazard classification of chemicals. Dr. Stokes said this was an issue more technical than the typical issues brought to SACATM for the group's input. However, as an *ad hoc* issue relevant to the evaluation of the validation status of *in vitro* test methods for regulatory safety testing, it was considered important to bring it to SACATM's attention, and to gain SACATM's perspective on the scientific analyses and questions involved. He emphasized that NICEATM and ICCVAM are not asking SACATM for a decision on whether GHS should be accepted, because the U.S. has already agreed to implement GHS, but rather to obtain SACATM's feedback on the appropriateness of the data analyses and conclusions. Dr. Freeman asked for clarification on the objective of the three proposals. Dr. Stokes said the proposals were drafted as three options for updating the GHS to allow for hazard labeling categories that could be used by the U.S. and other countries to maintain the same level of hazard labeling as currently required by their national safety regulations, consistent with the GHS principles. Dr. Corcoran asked about the EU system in relation to the systems used by EPA in the U.S. and by Health Canada, which are more protective. Dr. Stokes replied that there are no data available to assess the effectiveness and level of protection afforded by different national requirements, due to gaps in the eye injury reporting system currently in place. Dr. Corcoran clarified that he was interested in the animal testing data, which apparently is significantly less sensitive and protective under the EU system. Dr. Kreysa replied that GHS is actually somewhat more protective than the older EU system, which apparently had not resulted in any increase in eye injuries due to classification of substances as non-hazards, although he acknowledged that there is not a systematic monitoring system in place or other data to confirm this.

B. Public Comment

Karen Barlet, Monroe, North Carolina, shared her story of having been in a serious automobile accident in 2002. The deployment of the air bag in her vehicle resulted in her eyes being burned by the chemicals in the air bag propulsion system, leading to severe eye injuries. After many operations since the accident, she ultimately lost her left eye, and her right eye continues to deteriorate and would eventually also need to be removed. Although she had high praise for her caregivers, particularly Drs. Craig and Amy Fowler, she urged the committee to bring more attention to the danger of serious eye injuries associated with the chemicals in air bags, and the importance of warnings for consumers about the presence of chemicals that can cause eye injuries. She also advocated educating first responders to be aware of the danger, which would allow them to remove victims from vehicles more quickly and treat their potential eye injuries more effectively. Drs. Brown and Freeman expressed their sympathy to the speaker and thanked her for sharing her story.

C. SACATM Discussion

Dr. Proia, Professor of Pathology at Duke University and *ad hoc* discussant, praised Dr. Haseman's statistical analysis, and recommended strongly against adoption of the current GHS standards. Although the rabbit is not a good model for human injury, it is exquisitely sensitive, he said, and he would not embrace weakening standards.

Dr. Peiffer, veterinary ophthalmologist at Merck and *ad hoc* discussant, concurred with Dr. Proia that the Draize test is flawed, subjective and crude, but the only standard currently available. He opposes reducing the current requirement to a three-animal Draize test due to the potential for greater likelihood of false negatives. Dr. Proia cited the variability of eye injuries and among individuals, and the difficulty of defining clinical relevance. Dr. Peiffer said he found it especially concerning that the GHS system missed some EPA Category 2 compounds, and that there is no question that one would not want to expose one's ocular surface to one of those compounds.

Dr. Proia felt that it would be dangerous to remove hazard labeling from any of the currently labeled chemicals, as it would lead people to become more lax in their handling of the substances and would likely lead to more eye injuries as a result. Dr. Peiffer said there was a lack of human eye injury data, and urged that a mechanism be established to gather that information, possibly a reporting system with ophthalmologists, who would see many of the patients with such injuries. He said the trend should be toward a system that is more, rather than less protective, and he endorsed any of the three proposed updates to GHS on that basis.

Dr. Proia said that in his experience *in vitro* methods were extremely complex, and that we are likely decades away from developing effective assays to replicate *in vivo* situations accurately and reliably. Dr. Freeman responded that *in vitro* assays validated to assess eye irritancy exist today, but focus on the classification issues rather than seeking to address biological questions. Dr. Stokes added that existing assays are capable of predicting some of the substances that can cause irreversible eye injuries, and have value on that basis. Dr. Peiffer suggested prospective studies involving

improved animal models in comparison to *in vitro* approaches, although that would necessitate more animal use. Dr. Proia reiterated Dr. Peiffer's comment on the lack of human ocular injury data. Dr. Stokes explained that the question sought to determine whether there would be any value in seeking more detailed information using modern ophthalmic instruments, as opposed to the subjective observations currently in use. Dr. Peiffer said the Draize test could certainly be refined to make the data more valuable. Dr. Proia said histological correlates with the changes observed in the Draize test would be helpful. Dr. Peiffer suggested measuring corneal thickness, and Dr. Proia suggested confocal microscopy to detect cell death.

Dr. Corcoran, lead discussant, felt that population variance would make the three-animal test difficult to accept. He agreed with Drs. Proia and Peiffer that a zero tolerance policy was called for, but expressed some hesitation in terms of the costs involved and the burden of regulation. Being overly protective, however, would support the EPA's approach of assessing risks to the most vulnerable populations. He was concerned about confounding with existing databases, and said he would be more comfortable assessing the range of injury along with the ranges of other effects.

Dr. Hansen, lead discussant, concurred with much that had already been said, but pointed out that he believes the average consumer does not understand the difference between the words *danger*, *warning*, and *caution*, which are used in EPA eye hazard warning categories. He considered labeling addressing treatment as far more important than the classification categories.

Dr. Olson, lead discussant, said that he found the 33% positive rate in the three-animal assay to be acceptable, but that two blinded readings of the assay may be necessary to avoid bias. He agreed with Dr. Hansen's remarks about the importance of first aid information being included on labeling, with a note to seek professional help after exposure being added to the labeling of the more dangerous substances.

Dr. Qu, lead discussant, said there should be more concern about false negatives than false positives in this area. She suggested using both eyes in the rabbit test, which might remove the confounding presented by individual variation within a population.

Regarding the availability of human data, Mr. Wnorowski asked Dr. Fowle about an EPA database of adverse human effects from compounds in the market. Dr. Fowle replied that EPA had looked closely at that database as a potential source of human data in this area, but found it to be lacking in a variety of ways that rendered it unusable. Dr. Toth asked about the attention paid to reversibility in the Draize test, given that the eye is already damaged, regardless of whether the injury is reversible, and why these studies could not be terminated after injuries are observed. Dr. Stokes replied that earlier humane endpoints had been proposed to the peer review panel that met in 2009, but there had been some reluctance to adopt some of the earlier endpoints because some of the injuries might actually reverse within the 21 day observation period. However, he noted that in some cases, when permanent damage is unequivocal, this could be used to stop a test. Mr. Wnorowski, lead discussant, concurred, stating that very often in

laboratories, studies are terminated as soon as a very severe reaction is seen, regardless of when it occurs.

Dr. Meyer expressed concern that the discordance between GHS and current U.S. standards would continue as new compounds are introduced. Dr. Stokes said that GHS will be adopted, so that would not be an issue. Dr. Meyer asked if that meant a reduction of standards. Dr. Stokes said that this would occur if GHS were adopted as currently written. However, there is the opportunity for the U.S. to utilize GHS procedures to request updating of the GHS to add an optional category that could serve to negate the reduction in protection. Dr. Stokes explained some of the differences between the U.S. and GHS standards, particularly in terms of what would be considered positive responses in animals. He elaborated on ICCVAM's responsibility to provide scientific data and analyses to agencies that can assist them in determining whether new methods are effective in generating data that does not result in less protection than existing test methods. Dr. Fowle said the Harmonization Act refers to harmonization of test protocols, not classification schemes, which gets at policy and risk assessment. Dr. Fowle said it is not the role of ICCVAM to address classification schemes, only test protocols.

June 18, 2010
Dr. Freeman reconvened the meeting at 8:30 AM. Attendees introduced themselves and Dr. White read the conflict of interest statement.

IX. Updates on International Collaborations

A. European Centre for the Validation of Alternative Methods

Dr. Joachim Kreysa showed a promotional film made by the European Commission's Joint Research Center Institute for Health and Consumer Protection on protecting the European consumer and the use of science for a healthier life. The film demonstrated ECVAM's promotion of the development and dissemination of alternative methods to replace animal testing of consumer products. Dr. Sharon Munn, ECVAM coordinator, and other scientists describe the work done using human cells from umbilical cord blood, nanotechnology, advanced computational methods, high throughput systems, and the ECVAM databases.

Dr. Kreysa noted the assays for which validation is completed, but that are still in the regulatory acceptance process: the rLLNA, ICCVAM-ECVAM-JaCVAM harmonized LLNA Performance Standards (included in the revised OECD TG 429), three *in vitro* skin irritation tests (Epiderm™, Episkin™, and Skin Ethic™), the Guidance Document on using *in vitro* cytotoxicity tests to estimate starting doses for acute oral systemic toxicity, the Draft TG on *in vitro* micronucleus for genotoxicity, and the Guidance Document on application of the threshold approach for acute fish toxicity testing. TGs are in preparation by ECVAM for two cell-based assays for eye irritation, the Fluorescein Leakage Assay and the CM Assay.

Validation has recently been completed for the 3T3 Neutral Red Uptake for acute oral toxicity. Reports and publications in preparation for pre-validation studies on three cell transformation assays for carcinogenicity. ECVAM is currently involved in the validation of 21 assays (seven of which are ECVAM-led or coordinated) including (1) eye irritation **–** Epiocular™ and Skinethic™; (2) skin sensitization **–** prevalidation study on three partial replacement methods; (3) genotoxicity/mutagenicity – prevalidation of micronucleus and comet assays in reconstructed human skin models and validation of *in vitro* and *in vivo* comet assays; (4) carcinogenicity – cell transformation assay; (5) toxicokinetics/metabolism – hepatic biotransformation enzyme induction and metabolic competent test system; (6) reproductive toxicity – three ER transcriptional activation assays; (7) ecotoxicity – zebrafish embryotoxicity and *in vitro* S9 trout assay. ECVAM must set priorities as to which of the ten to twenty test submissions per year would be validated.

ECVAM is aware of several known or expected test submissions including ER transcriptional activation assays, an androgen receptor transcriptional activation assay, a genotoxicity assay, and a neurotoxicity assay. In most cases these methods would not be replacement methods but "building blocks" for testing strategies**.** There are also some sufficiently similar ("me-too") methods for skin and eye irritation that can be validated more easily if performance standards are clear. Other ECVAM activities include (1) reduction of false positives in genotoxicity testing, (2) ECVAM's new 2-step test submission procedure, (3) the first meeting of the renewed ESAC, (4) ECVAM's report on alternatives for cosmetics for the 2013 deadline, (5) ECVAM's DB-ALM database service, (6) ECVAM technical reports uploaded on the ECVAM website, and (7) the ECVAM validation process.

Dr. Kreysa then described the seven steps involved in the progress of an ECVAM recommendation which are (1) test submission handling, scientific/relevance assessment and optimization; (2) planning and conduct of validation studies; (3) ECVAM request to ESAC, ESAC review and opinion on the study conduct, conclusions, and validity; (4) draft ECVAM recommendations; (5) "right to be heard" process; (6) public commenting; and (7) finalization and publication of ECVAM recommendation, including ESAC opinion and validation study report. He further described the involvement of ICATM and the EU member states in the stages of the validation process.

Dr. Kreysa explained the role of ECVAM in the European Partnership on Alternative Approaches to Animal Testing, which is a public-private partnership between all relevant services of the European Commission and seven industrial sectors, both trade Federations and individual (multinational) companies. Participation is voluntary in the Partnership, with the goal of promoting development and application of methods for the 3Rs in three key areas, science, regulation, and dissemination.

Dr. Ehrich asked where the film would be shown. Dr. Kreysa responded that it would be shown at exhibitions and conferences. Dr. Bucher congratulated Dr. Kreysa on his outstanding efforts to make the international cooperation work and to make the

processes move more quickly. Dr. Stokes added his thanks and acknowledged the efforts of ECVAM staff and management in making ICATM successful. The effort would pay off in the future with faster adoption of validation methods and increased transparency. Dr. Kreysa said it is a mutual activity and compromises must be found when necessary. Dr. Kreysa responded to Dr. McCormick that students at Karolinska Institute and the University of Brussels are taught about alternative methods. Dr. Olson asked about the plans for ECVAM's reformed ESAC. Dr. Kreysa said there would be a minimum of two meeting per year; it would depend on the workload. The bulk of the work of ESAC would be peer reviews, which will start this fall. WGs will convene between regular meetings to review the background material and create draft opinions, on which ESAC would vote in plenary sessions.

B. Health Canada

Dr. Blakey said in 2007 Health Canada was invited to join ICCVAM, ECVAM and JaCVAM in the cooperative approach to validating alternative test methods and reaching consensus on recommendations regarding their suitability for use. He regards this as incredibly important because the people who use these tools and interpret the data, do not easily understand in all cases their suitability. They receive a lot of new information and there is a challenge in trying to understand whether a new test is acceptable for regulatory purposes or any other purpose. This is a valuable contribution that ICATM can make to the regulatory scheme and to the adoption of alternative test methods. He considered it important, in going away from single stand-alone replacement tests to a more integrated approach, that alternative tests are put in the right context to get the best use from them.

Canada has no center for validation, as the other member countries do; but they have contributed their expertise to various stages of validation internationally for a number of years. He said he is the OECD TG coordinator for Canada and has participated in the development of the validation guidance documents. Health Canada has participated in various validation processes over the years, with their primary interest being to make sure alternative test methods and strategies offer equivalent or better protection for human health, while at the same time respecting the principles of the 3Rs and moving as quickly as possible to the replacement of animal tests. This activity in Canada is coordinated by the Environmental Health and Research Bureau, which conducts research in a number of areas, including analytical chemistry, biomonitoring, toxicology, mechanistic studies, and epidemiology. Health Canada is working along the continuum to attempt to close the loop from exposure through effect to human disease at the population level. Health Canada consists of 12 branches, offices, and bureaus and four agencies. The branch most involved in the regulation of harmful exposures is the Healthy Environments and Consumer Safety Branch, which deals with environmental contaminants and product safety. The Health Products and Food Branch deals with foods, drugs, and health products. There are a number of areas where alternative tests could be considered and play an important role in the protection of the safety of Canadians.

Over the years Health Canada has continued to contribute to ICATM's work, largely in pre-validation research, including method development and refinement. Projects include development a transgenic gene mutation assay, international collaborative studies on metal bioavailability/bioaccessibility, and International Programme on Chemical Safety (IPCS) collaborative studies. Health Canada continues to participate significantly in OECD validation management committees, including the mammalian and *in vitro* ED methods, and continues to assist with ICCVAM peer reviews. The activities on method development, refinement, and validation are mostly coordinated through OECD, IPCS, the International Workshop on Genotoxicity Testing, ICCVAM, and the Japanese Environmental Mutagen Society. Health Canada's role in ICATM would be to continue to contribute expertise for the validation of alternative tests. Health Canada is now building their capacity and would continue to contribute to validation management, the design of validation studies, review of validation studies, and the peer review process. They will cooperate on the recommendations that are provided for the use of these tests in the regulatory environment. Most of the work would be accomplished through review of documents and provision of expertise. The bureau is in the process of dedicating resources to the activities of ICATM and other guideline-related activities to allow acceleration of activities with ICATM, thus becoming a more valuable partner.

Dr. Blakey closed by reminding SACATM that Canada will host the 8th World Congress on Alternatives in Montreal in 2011. Dr. Stokes thanked Dr. Blakey and pointed out that Health Canada was one of the original charter members of the International Cooperation on Cosmetic Regulation (ICCR) and participated in the first meeting of the ICCR in 2007. There it was determined that there was a need for greater international cooperation. Canada helped with developing the framework that has evolved into the ICATM MOC. In 1995, Health Canada was involved in efforts at the original workshop for development of criteria for validation of methods for regulatory acceptance.

C. Korean Center for the Validation of Alternative Methods

Dr. Han provided a report on the current status of alternative test methods in Korea. Korea has several organizations dedicated to protecting animal rights and welfare and to raise awareness of the importance of animal protection. Several cosmetic companies and contract research organizations (CROs) have shown an interest in studying alternative test methods and are preparing to be actively involved in research on alternatives to advance into the European market. Several academic societies are interested in alternative test methods, such as the Korean Society for Alternatives to Animal Experiments (KSAAE), which has held a number of symposia and issued publications to share the information since 2006. The Seoul Satellite Symposium was jointly held as part of the 6th World Congress on Alternatives and Animal Use in Life Sciences in 2007.

Korea developed domestic guidelines based on OECD TGs for (1) phototoxicity testing – *In vitro* 3T3 NRU phototoxicity test (OECD TG 432), (2) skin sensitization testing – LLNA (OECD TG 429), (3) acute oral toxicity testing– Fixed Dose Procedure (OECD TG 420) and Acute Toxic Class Method (OECD TG 423), and (4) *in vitro* skin absorption method (OECD TG 428). Korean guidelines were developed so that researchers in

industry would have easy access to test methods. Companies can submit reports on tests that were conducted in compliance with guidelines to get approval of KFDA and/or to evaluate safety. Korea will continue to develop more guidelines.

Dr. Han said the National Institute of Food and Drug Safety (NIFDS) within the Korean Food and Drug Administration (KFDA) has held three workshops since 2007 on alternatives for skin irritation testing, eye irritation testing, skin sensitization testing, and phototoxicity testing, in which they worked with over 200 trainees. KFDA/NIFDS made efforts to develop alternatives to biological assays that are used for lot release and/or other quality tests. *In vitro* assays for hepatitis B vaccine potency and an identity test for botulinum type A toxoid were developed and established. Both tests are routinely used for national lot release and manufacturers' quality control testing. Molecular methods for quantifying mycoplasma and retroviruses were also developed. Currently, KFDA is developing an *in vitro* assay for Japanese encephalitis vaccine.

Dr. Han showed a photograph of the Memorial Tower for Laboratory Animals. A ceremony is held every fall to commemorate animals sacrificed for improved understanding and protection of human health. She described the establishment of KoCVAM in the KFDA in November 2009 in Seoul, Korea. The legal basis for KoCVAM's establishment is the Laboratory Animal Act, signed on March 28th, 2008 to establish policies and their execution on the development and approval of alternative test methods, which are specified as duties of the commissioner of KFDA. She described the organization of KFDA, in which KoCVAM is in the Toxicological Evaluation and Research Department within the NIFDS. KoCVAM is responsible for (1) promoting the 3Rs in regulatory science for the safety assessment, (2) evaluating the usefulness and limitations of alternative test methods, and (3) promoting cooperation both nationally and internationally to achieve international harmonization. KoCVAM has a steering committee, a scientific advisory committee, and three *ad hoc* teams including a validation management team, a peer review team, and a guideline advisory team. KoCVAM interacts with OECD, ICH, ISO, ICCVAM, ECVAM, and JaCVAM and it will continue to promote cooperation both nationally and internationally to achieve international harmonization and to facilitate international acceptance of new and revised test methods. KoCVAM held an international symposium on November 3, 2009 at Seoul National University in which the directors of ICCVAM and JaCVAM participated. The symposium addressed global efforts for regulatory acceptance of alternative test methods, the validation process of test methods in Korea, and applications of innovative technologies for safety assessment. The first KoCVAM workshop, titled *Understanding of OECD Guidance*, was held on February 10, 2010. To introduce the new science of alternatives to the Korean public, an article was published in a daily newspaper titled, *Eggs save rabbits from painful experiments*. KoCVAM is using a number of approaches to promote alternatives including (1) participating in international validation studies, such as the CCi MCF-7 cell proliferation assay, the stably transfected transcriptional activation assay, and the *in vitro* comet assay; (2) conducting exploratory studies to develop alternative test methods; and (3) disseminating OECD TGs in Korea by working together with CROs, academia, and industry to develop domestic TGs.

Dr. Han described the KFDA contributions to the development of OECD TGs TG440, TG441, and TG455 for EDs. Ongoing exploratory studies include (1) LLNA: BrdU-FC, (2) LLNA performance standards, (3) data on 18 reference substances, and (4) inter-laboratory and intra-laboratory validation using 2 reference chemicals by three laboratories. KoCVAM is disseminating OECD TG 487 (the *in vitro* micronucleus assay) to six CROs and OECD TG 437 (the BCOP) to two CROs and one academic institution, and is working on the preparation of domestics TGs.

Dr. Han closed by saying that Korea is in the early stages of developing alternatives, but KoCVAM is hoping for more opportunities work with other international validation organizations to learn and share knowledge about alternatives. Dr. Brown said KoCVAM is using a good approach to publicize the use of alternatives to animal testing; educating the public is very important. Dr. Ehrich complimented Dr. Han on how much KoCVAM has accomplished in such a short period of time. Dr. Stokes added that ICCVAM-NICEATM has developed a close working relationship very quickly with KoCVAM. He appreciated their enthusiasm for participating in the joint validation study; it was a great example of how different validation organizations can leverage resources. Having validation studies conducted in different parts of the world is a good way to demonstrate the reproducibility of those methods and provide much more confidence in the validity of those methods.

X. Alternative Methods of Vaccine Potency Testing

A. *In Vitro Leptospiral* Bacterin Potency Testing

Dr. Kulpa-Eddy discussed development of *In Vitro Leptospiral* Bacterin Potency Tests at the UDSA. She described the taxonomy of the bacteria and explained that *L. interrogans* is the pathogenic species. Pathogenic leptospires are not readily distinguishable on the basis of morphology, biochemical, or cultural characteristics, but they do have distinctive antigenic properties that can be demonstrated serologically using the microscopic agglutination test (MAT). Leptospires occur naturally in a wide variety of feral and domestic mammals and cycle in the environment through natural (maintenance) hosts that include rat, raccoon, dog, sheep, and swine. A zoonotic disease occurs when incidental hosts (i.e., humans) are infected through occupational or recreational activities involving direct contact with infected urine, or contact with water and/or soil contaminated with infected urine. Clinical manifestations are variable and depend on whether the animal is a natural or incidental host. Other factors include the exposure dose, route of exposure, immune and hormonal status of the animal, pathogenicity of the inocula, and previous exposure. Symptoms in maintenance hosts are usually not apparent; however, in incidental hosts the acute phase includes flu-like illnesses, hemolytic anemia, hemoglobinuria, and jaundice. In the chronic phase in definitive hosts there is kidney and liver damage. Leptospirosis in animals is controlled by vaccination to provide a barrier against human exposure. Immunity is generally humoral, but there is a cell-mediated immunity component.

The USDA Center for Veterinary Biologics (CVB) is responsible for ensuring that products are
not contaminated, not dangerous or harmful, and not worthless (i.e., the CVB is ensures purity, safety and potency/efficacy of products). Currently leptospiral vaccines are

tested with the hamster vaccination-challenge assay, as outlined in the Title 9 CFR. For this test hamsters are vaccinated with a specified dilution of the bacterin and then exposed to virulent challenge with an appropriate serovar 14 days later. After 14 days, the number of live and dead hamsters are determined. A minimum of 80% of vaccinates must survive and a minimum of 80% of controls must die. The disadvantages of this testing include the use of a large numbers of hamsters, a great deal of time and labor, and exposure of personnel to viable pathogenic organisms. The proposed potency test to replace the vaccination-challenge assay is the ELISA that uses monoclonal antibodies prepared against host animal virulent cultures. The advantages of the ELISA are that it measures a relevant antigen, uses no hamsters, promotes the 3Rs, is less expensive ($640/hamster test compared to $2/ELISA, based on FY 2001 data), is faster, and personnel are not exposed to a human pathogen. She described the test development which began in 1991 to first identify the relevant antigen, which is key for ELISA testing. In 1998 the CVB issued the *Guidelines for Veterinary Biological Relative Potency Assays and Reference Preparations Based on ELISA Antigen Quantification*. In 2000 the CVB released the Supplemental Assay Methods developed for *in vitro* potency testing of *Leptospira* serovars and in 2002 released a memo issued regarding *Exemption from Leptospira Bacterin Testing Under 9 CFR* to communicate to industry that the USDA was open to accepting these alternative test methods. Further development and validation of the ELISA, including dog efficacy tests, continued through 2007, when the CVB issued two guidance documents, *Qualification of Leptospira grippotyphosa and Leptospira icterohaemorrhagiae Reference Bacterins for Products Intended for Use in Dogs*, and *Qualification of Leptospira pomona and Leptospira canicola Reference Bacterins for Products Intended for Use in Dogs*. In 2008 pig efficacy studies were completed and the CVB issued *Guidelines for Validation of In Vitro Potency Assays*. In 2009 the CVB issued *Qualification of Leptospira Canicola, Leptospira Grippotyphosa, Leptospira Icterohaemorrhagiae,* and *Leptospira Pomona Reference Bacterins for Products Intended for Use in Swine and/or Cattle*. A remaining hurdle is that adjuvants or other components may interfere with the assay. The total time to develop the replacement ELISA test was approximately 19 years at a cost of approximately $2 million. The net effect of the development of this *in vitro* assay is that the number of hamsters used is decreasing while the number of doses of vaccine is steady or increasing. Dr. Kulpa-Eddy said that trend should continue.

Dr. Niemi asked if a manufacturer can submit potency testing data that is totally from *in vitro* assays. Dr. Kulpa-Eddy said yes, if a qualified reference bacterin is used. Dr. Kulpa-Eddy responded to Dr. Elmore that previously 30 hamsters per serovar were used. Dr. Toth asked if the use of any other species has been reduced similarly. Dr. Kulpa-Eddy said a 2000/2001 review of animal usage reported as painful/distressful listed hamsters as #1, followed by guinea pigs (skin sensitization and human vaccine testing) and rabbits (skin irritation). No follow-up to that review has been done recently. Dr. Andrews was very impressed with the work and asked for the correlation between an ELISA, which is a binding assay, and the *in vivo* assay potency assay. Dr. Kulpa-Eddy said the term “potency” may be used too loosely, but the ELISA verifies that product is not worthless.

Dr. Brown thanked the USDA and Dr. Kulpa-Eddy for all the work that has been done. She provided additional background on development of the *Leptospirosis* potency testing. To license any new *Leptospirosis* vaccine, companies had to do a host animal immunogenicity study, which involves vaccinating host animals and challenging them to demonstrate protection in the host animal. The results had to be correlated to the hamster test. Companies found that the antigen by itself will actually protect hamsters, and the adjuvants would improve the immune response, providing a much higher response in host animals than in the hamster tests. The hamster test is a more stringent test compared to the host animal test. More than 10 companies had already compared the host animal test with the hamster potency test and all data were submitted to the USDA. At the start of USDA *Leptospirosis in vitro* test development, industry began working in developing *in vitro* assays with the goal of being able to discontinue hamster potency testing. In 2009 some additional requirements were added, causing companies to return to the hamster tests because of the validation requirements that are required to keep their own master references going. She said the problem with these *in vitro* tests is that the USDA has required that there be additional monitoring of companies' master references to demonstrate that they are stable. This additional validation every three to five years doesn't eliminate animal testing and has sometimes increased the use of host animals. A similar issue occurred with feline leukemia, for which an early *in vitro* assay was developed. The references had to be revalidated in cats every three years, and it takes a year to run a feline leukemia study. Companies were running continual cat vaccination challenge studies, each of which require 60 - 120 cats per test. She advocated for an archive of existing industry data in the USDA to be used to facilitate development of *in vitro* tests to save time and money in test development. For products like *Leptospirosis* vaccines, where 9 CFR tests have been correlated to host efficacy tests, the tests should be used to qualify and requalify a master reference, which would save a considerable amount of time. Dr. Brown said companies would not pay to rerun their host immunogenicity tests, especially with inexpensive vaccines. This industry cannot afford to do extensive validation of every vaccine. She further advocated for the USDA and industry to continue to work together on developing *in vitro* assays, to assess the assay validation and monitoring requirements, to reach agreements which would limit the number of tests that have to be done, and to provide guidance regarding test acceptance to avoid rejection of test results. Dr. Kulpa-Eddy thanked Dr. Brown for her perspective, encouraged industry to release information, and reminded attendees that the upcoming workshop should address some of these issues. Dr. Kreysa said at their recent workshop on vaccines, a consistency approach was advocated, which included good quality control of the vaccine production process to reduce potency testing. Industry is forming two platforms, one for the animal vaccines and the other for the human vaccines, which would be used together with *in vitro* analyses.

B. International Workshop on Alternative Methods to Reduce, Refine, and Replace the Use of Animals in Vaccine Potency and Safety Testing: State of the Science and Future Directions

Dr. Richard McFarland, FDA/Center for Biologics Evaluation and Research, provided information on the vaccine potency and safety testing workshop being held September 14-16, 2010 at the William H. Natcher Center, Bethesda, MD. The workshop builds on the 2007 *Botulinum Toxin International Workshop* and involves many of the same people. The FDA is seeing positive interactions with outside groups and industry regarding issues of regulatory use. Structured like the 2007 workshop, the 2010 workshop will have plenary sessions and daily breakout discussion groups. Information on the workshop is at *http://iccvam.niehs.nih.gov/meetings/BiologicsWksp-2010/BiologicsWksp.htm.* The rationale for the workshop is (1) biologics testing is one of ICCVAM's four highest priorities because it can require large numbers of animals that may experience significant pain and distress during testing, and is required by multiple agencies; (2) it is important to identify *in vitro* alternatives to the current *in vivo* tests that provide equal or greater protection of human or animal health, and to identify procedures that can be used to reduce or avoid pain and distress where animals must still be used; and (3) alternative test methods are under development that target reduction and replacement of animal testing with *in vitro* test methods, as well as refinement of animal testing through modifications to the current animal tests. The goals of the workshop are to (1) review the state of the science of currently available alternative methods that reduce, refine, and replace animal use in vaccine potency and safety testing; (2) identify knowledge and data gaps that must be addressed to develop new alternative methods; and (3) identify and prioritize research, development, and validation efforts that will address these knowledge and data gaps to advance alternative methods for vaccine potency and safety testing. The workshop objectives are to (1) review the public health needs and regulatory requirements for vaccine potency and safety testing; (2) review the currently available and/or accepted alternative methods that reduce, refine, and replace the use of animals for vaccine potency and safety testing; (3) identify and discuss the current development and/or validation status of proposed alternative methods; (4) identify knowledge/data gaps and prioritize future research, development, and validation initiatives to address these gaps; (5) discuss how to promote the collection/sharing of data to advance the development and validation of alternative methods; and (6) discuss ways to promote international harmonization and/or acceptance of vaccine potency and safety requirements, including the acceptance of alternative methods.

Dr. McFarland then listed the invited speakers, which included a range of U.S. government and industry scientists, as well as many international scientists. He provided an overview of the workshop program. Titles for the sessions are (1) *Overview of Public Health Needs and Regulatory Requirements for Vaccine Safety and Potency Testing*, (2) *Replacement Methods for Vaccine Potency Testing: Current State of the Science and Knowledge Gaps*, (3) *Animal Use for Vaccine Potency Testing: Refinement and Reduction Alternatives*, (4) *Vaccine Post-licensing Safety Testing: Reduction, Refinement and Replacement Methods and Strategies.* Breakout groups will address the topics (1) *Non-animal Replacement Methods for Vaccine Potency Testing: Current State of the Science, Knowledge Gaps, and Research Needs*,(2) *Methods and Strategies for the Refinement and Reduction of Animal Use for Vaccine Potency*

Testing, and (3) Vaccine Post-licensing Safety Testing: Reduction, Refinement and Replacement Methods and Strategies.

Dr. McFarland then listed the questions drafted by the organizers for the breakout groups. The workshop organizing committee is the ICCVAM Biologics Working Group (BWG), which consists of representatives from the CDC, USDA, Department of Defense (DoD), Department of Interior, EPA, FDA, NIH-National Institute of Allergy and Infectious Diseases (NIAID), NIEHS, Health Canada, ECVAM, and JaCVAM. Drs. Kulpa-Eddy and McFarland co-chair the BWG.

C. SACATM Questions and Discussion

Dr. Birnbaum urged having more involvement from the NIAID in the BWG and on the program because NIAID develops vaccines. She mentioned the recent editorial by Drs. Collins and Hamburg on increased interactions between NIH and FDA on translational research. Dr. McFarland concurred and said increased interactions are occurring. Dr. Stokes said the CDC is very involved in vaccine development for different strains of influenza. Names not listed in the presentation are becoming involved and would participate in the workshop. Dr. Meyer said the DoD should be included because of their work in the Countermeasures Against Chemical Threats (CounterACT) Program. Dr. Birnbaum said NIEHS in involved and NIAID has the lead for NIH in CounterACT. Dr. Stokes said there are two representatives on the organizing committee from the U.S. Army Medical Research Institute for Infectious Diseases. Dr. David Allen said there is no registration fee for the workshop and registration is on the front meeting page. Dr. Diggs said graduate students, postdoctoral fellows, veterinary residents should be encouraged to attend as a way to inform new scientists.

Dr. Brown said she was looking forward to the workshop and suggested including references from the FDA on developing human vaccines to help with veterinary methods development. An organization within USDA could be formed to develop some of the references for the whole industry. If single references were kept stable and validated at the government or an institutional laboratory, then the industry itself can monitor its own internal references against that, avoiding the need for using animals. It is doable, but it would take everyone collaborating.

Dr. McCormick asked how much information the USDA gets on adverse reactions to vaccines. Many laboratory beagles have adverse reactions to the Leptospira bacterin, so her company does not vaccinate beagles. She questioned whether safety would be addressed at the workshop.

Dr. Stokes encouraged graduate students and postdoctoral fellows to participate in the poster sessions at the workshop and Dr. McFarland pointed out the suggested readings listed in the notebook and on the website.

Dr. Laura Andrews, lead discussant, said biologics, and vaccines in particular, are difficult to develop. She agreed with Dr. Brown's suggestions on what needs to be done. She suggested a number of things to be kept in mind in the context of the 3Rs. It is important to work collectively to ascertain what is the right assay to use for the right

question, which is not done very well right now. There is a tendency to do things like they were done in the past because they worked. She agreed that it is very daunting to do the work of developing the *in vitro* assays, but there are ways of collectively or individually working on them. She said it was important to fully understand what potency is, so discussing potency would be a beneficial discussion at the workshop. She advocated talking with people to leverage the information, using databases, and opportunity-sharing to advance the science. Asking the right questions and sharing information would allow reductions in the numbers of animals and provide a path forward. It is important to challenge the relevancy of the species and tests used and not just use what is available. Another question to be asked in the breakout groups is about the safety of the new adjuvants and techniques used. Moving forward must include the totality of the data using a tiered approach with initial screening for potency and safety, followed by a really good study using animals only if necessary. Dr. Andrews said biologics can be developed without the use of animals

Dr. Brown, lead discussant, concurred with Dr. Andrews. She reiterated the need for interaction, scientific discussion, and cooperation between the government agency and industry.

Dr. Elmore, lead discussant, agreed that development of reference standards is important, especially for inexpensive vaccines. Industry must be made to realize, in a positive light, the importance of sharing the data as a cost-effective way of improving their assays, reducing costs, and increasing profits.

Dr. McCormick, lead discussant, suggested taking the lead from the cosmetic industry in Europe and setting a goal to in 20 years eliminate 50% of the animals we use now. That may be an incentive for the manufacturers and the agencies to work together to make progress in this area. It will be while before we can totally eliminate the use of the animals. Anything that can be done to minimize pain and distress in animals by refining endpoints will aid in the process.

Dr. Niemi, lead discussant, commended the CVB for their progressive approach on the ELISA and suggested whether it would be better to use a species-specific polyclonal antibody to show protection for that species, and then use a monoclonal mouse antibody as the secondary antibody. Regarding CFR testing, he suggested the USDA make *in vitro* testing the default, which could eliminate hamster usage. In the meantime, eliminate animals at each time point for which the alternative *in vitro* assay becomes approved, with the caveat that the *in vivo* assay would be the backup if the reagents that are provided or reviewed by the USDA are of insufficient quality or quantity. Regarding SBIRs and replacement assays for vaccine testing, this is an opportunity for small companies to develop something proprietary that would be marketable, leading to immediate revenues. The USDA should also consider Cooperative Research and Development Agreements with larger companies to develop these methods. The scope of the September workshop should be expanded to include antisera because many of the same potency, testing, and pre-release evaluation assays are similar. To encourage manufacturers to submit alternative methods for vaccine

potency assays, tangible incentives should be provided, modeled after the Prescription Drug User Fee Act of 1992, that provided faster review times of new pharmaceuticals by the FDA. Vaccines are a low margin business, so expedited approvals may be economically important to the manufacturer. Sharing negative control animals between a group of assays may save money (and animals) as well. Regarding humane endpoints, he advocated looking at the kinetics of the microbe in the bloodstream or in the targeted tissue after challenge and then create a curve that would predict death at a later time. One may then develop assays, based on these more informative data regarding pathogenesis, where the animal's life is terminated even before clinical signs are manifest. He added that it is important to be aware of the normal gut flora in laboratory animals in terms of how they may affect immune endpoints.

Dr. Brown said some companies cannot afford the cost of development of *in vitro* assays, and have to take products off the market because of the cost of the revalidation and requalification of references and the continued monitoring of stability of the references. Dr. Meyer expressed surprise that an *in vitro* test is more expensive than an animal test. ICCVAM should look at the cost effectiveness of alternatives before they get to the translational stage. Dr. Stokes pointed out that when ICCVAM proposes a new alternative method, they look at practical considerations, including cost. If a method is so costly compared to the existing method that is not going to be used, then it wouldn't make sense to go forward with it. He agreed with Dr. Niemi regarding humane endpoints and said the goal was to find earlier, more humane, mechanism-based endpoints. Dr. Freeman said there is a perception issue regarding cost, which would be high due to development of new technology, building capitol, and additional training. The older standard toxicology tests are rote and the costs have been driven down. For some of the alternatives, the costs may go down and some would have hidden costs that weren't initially realized. Dr. Brown said it is costing each company $2 million for each Leptospira *in vitro* test development. Actually conducting the assay can be less expensive; but it takes time to make up the cost. Dr. Kulpa-Eddy clarified that the $2 million price for Leptospira is not true anymore now that the USDA has done the work, and that would keep costs down for each company. Dr. Brown agreed, but said that starting from scratch on a new *in vitro* assay costs $1 - 2 million and take 5 -15 years, similar to the time for developing a new drug.

XI. Other Business

SACATM returned to the issue of pain assessment and alleviation. Dr. Corcoran felt this was an issue the committee felt strongly about, and he prepared a statement conveying his sense of SACATM's views, while avoiding the term "priority." He stated a motion: *Recognizing the significant unmet need for increased understanding and for improved methods to assess and alleviate pain in animals used in research and testing, SACATM recommends that these be given increased research and development attention and commensurate support.* Dr. Corcoran said the intent of the motion was to provide ICCVAM and NICEATM with an expression of SACATM's support for efforts to move the concept forward. Dr. Diggs seconded the motion. Drs. Meyer and Ehrich asked if the recommendation really needed to be a motion and Dr. Meyer asked how funding might be put in place to support a prioritization. Dr. Bucher said NICEATM, like

any other organization, is working within a budget to support its currently identified priorities, which inevitably shift and evolve over time. Dr. Stokes said within ICCVAM-NICEATM's FYP, there is a reference to the need for flexibility in terms of priorities; they would change as needed to take advantage of new opportunities for progress or new needs that are identified. Prior to adoption, Dr. Olson said he would like to see the language of the motion sharpened a bit in terms of identifying how the increased attention and support would be directed. Dr. Corcoran replied that he had intentionally left those items out of the motion, to not inherently exclude any options.

Dr. Meyer asked about the practical consequences of the motion, if adopted. Dr. Stokes replied that it would be useful as the NTP and ICCVAM might decide how to go forward, and as they might approach other parties for additional support and resources to carry out such an activity. It would be very helpful to be able to convey to others the sense of SACATM's opinion.
Dr. Snyder said NIH is currently emphasizing cross-institute cooperation, and that National Institute of Dental and Craniofacial Research, with its long history of extensive research in the area of pain, might be a natural candidate for fruitful collaboration in this area. SACATM voted on the motion: 12 yes, 0 no, and 2 abstained. Dr. Olson said his abstention was based on not understanding the process involved in the meeting arriving at such an *ad hoc* outcome, and that his abstention was procedural and not based on any reservations about the intent of the motion itself. Dr. Toth abstained for similar reasons. Dr. Freeman said it was appropriate to bring the issue to a vote, but questioned whether that was within the bounds of his prerogatives as chair. Dr. Stokes mentioned that typically when ICCVAM-NICEATM might need a specific decision from SACATM, it would be stated clearly as an action item in the meeting agenda, but in this case the issue was not sharp enough as the meeting was being planned to approach it in that manner.

Dr. Fowle clarified questions from the previous day regarding EPA acceptance of the LLNA. Since last January, the EPA has been accepting the radiolabeled LLNAs for pesticide formulations. The EPA is drafting a policy to accept the rLLNA for pesticide formulations because there are EPA and OECD guidelines. Right now the EPA is not accepting nonradiolabeled LLNAs, but would do so when OECD adoption occurs. He directed SACATM to the *Strategic Directions for New Pesticide Testing and Assessment Approaches* website, which shows EPA's approach for decreasing the use of animals. The EPA is taking action to transform their approach to pesticide risk assessment by taking advantage of what is known about the natures of the chemicals, such as QSAR information, to get hypothesis-based approaches that don't have to rely on animal testing. He showed EPA's tools matrix, which consists of three tables, the first of which summarizes the work that is making the existing animal tests more focused on risk assessment. The second table shows activities to replace animal testing and the third table presents EPA's long-term approaches.

Currently, EPA is actively engaged in updating these approaches, which would be presented at a conference on November 16. There are five areas of focus, including more efficient animal and the ecological studies, because of the interest in species

besides humans. Other focus areas include chemical prioritization screening tools, animal replacement studies, risk assessment, and the implementation within the policies and the procedures. Dr. Meyer asked if industry and the contract labs can meet the time line to switch over to the new paradigms. Dr. Fowle said EPA has a Pesticide Program Dialogue Committee, with approximately 77 representatives from various sectors, including industry, environmental groups, and animal rights groups, to implement the steps sequentially to make sure the process would work.

XII. Closing Remarks and Adjournment

Dr. Freeman noted that this was his last meeting and thanked NICEATM and ICCVAM for the opportunity to chair SACATM. He said the work is important and there are challenges and opportunities coming. He was very heartened by seeing the gains in momentum he witnessed in the four years he spent on SACATM. He encouraged ICCVAM and NICEATM to make more transparent and harmonized the initiatives not just at Federal regulatory agencies, but internationally as well.

Dr. Bucher thanked everyone for an excellent meeting and said he appreciated the advice given by SACATM. This was an especially thoughtful round of discussions. Dr. Stokes reiterated his thanks and said the discussions were very insightful and productive. ICCVAM will use SACATM's advice for guidance in future activities.

Dr. Olson, noted his lack of familiarity with the SACATM process and asked about the possibility of teleconferencing prior to the meeting to discuss some of the content. He said if SACATM member knowledge could be shared prior to the meeting, it might stimulate members to do independent research and form opinions to bring to the meeting. Dr. Bucher explained that, under the Federal Advisory Committee Act, all discussions must occur in a public forum. Dr. Olson suggested other opportunities, in a public setting, where SACATM members have an opportunity to engage in discussions that would help provide deeper and more well formed opinions about the subject matter.

Dr. White announced the date of the next meeting, June 16 and 17, 2011, in the Washington DC area.

Dr. Freeman adjourned the meeting at 12:15 PM.